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Vol. 9 No. 1 *March 1957*

The Local Eugenics Society[1]

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THE TITLE of my paper, "The Local Eugenics Society", refers to a specific organization, The Minnesota Human Genetics League. The name, Human Genetics League, is technically more correct than the term Eugenics Society because the Human Genetics League would be willing, no doubt, to support work in human genetics which had no conceivable application to problems of human welfare. I would not ask the members to support any work unless there was a reasonable possibility that it would have significance for some of the genetic problems which perplex the thoughtful members of our world.

There is no question but that laymen who give their money to support human genetics research do so with the expectation that some findings from the research will eventually contribute something directly to the improvement of the genotype of our own species, Homo sapiens. These contributors are not fastidious as to the details of the research, nor will they try to dictate the choice of subject matter to be studied. However, it is my impression that projects concerned with the genetic and environmental factors affecting mental health will be very acceptable to any local Eugenics Society.

The laymen who contribute to a Human Genetics Society do so because they wish to make life better and happier for future generations, even though frequently they have no descendants themselves. As the childless person has made no genetic contribution to future generations, it is natural that he should be willing to devote some portion of his time and means to the organizations dedicated to an attempt to improve the future life on this planet.

Thus, in effect, gatherings of people who will promote genetic research, even if it is only with fruit flies and bread molds, are eugenics societies, regardless of what name they use, and even if they deny it.

There are some who think that any eugenics program must fail because the genotype of man is in such a rigid equilibrium that no appreciable changes could be brought about by humanitarian methods. Such people are mistaken. They cannot see into the future because their minds are shackled by the mistakes of some eugenics programs of the past. They fail to recognize that human society has carried out practical eugenics programs of defective gene elimination for centuries. At present persons with serious genetic defects, as well as those with serious environmental defects, are denied reproduction by being placed in institutions or by careful supervision of the sick person in the home. These methods are so universally acceptable that most people do not recognize their great importance in holding down our genetic load of deleterious mutations.

These people, who have been so preoccupied with the mistakes of the past, have aided and abetted the growth of the myth that any eugenics program must depend

[1] Presidential address delivered at the ninth annual meeting of the American Society of Human Genetics at the University of Connecticut, August 28, 1956.

upon some mechanical method of birth control and a violation of human rights, if it is to be effective. It is easy to show that this concept is false. The very neat demonstration by Allison that the elimination of malaria would in itself cut down the frequency of the gene for sickle cell anemia very appreciably in relatively few generations, is a case in point. Certainly no one can object to the eradication of the organism causing malaria and the automatic decrease of the frequency of the gene for sickle cell anemia which depends upon malaria for its present prevalence. Allison's research is a contribution to eugenics of the best sort, though he may not have thought of it as such.

It may be useful to re-emphasize my point that there is no important distinction between research in "pure" genetics and research in "applied" genetics such as eugenics. Our present day use of the term "human genetics" instead of "eugenics" may be financially and politically expedient but there is no great philosophical distinction between them. Indeed, "pure" science of any sort has no more reality than the "pure" race. Society supports and tolerates what is *called* "pure" science with the knowledge that the useless information discovered today will have value tomorrow. It is comforting to realize that even our mistakes may speed the discovery of the correct answers. Thus not much of our scientific energy is wasted in the long run. However, it would be wasteful to be complacent about our mistakes.

Let me give a short description of the local eugenics society with which I am well acquainted, the Minnesota Human Genetics League. The League is in reality the creation of a Minneapolis physician, Charles Fremont Dight, though it did not make its corporate appearance until some years after his death. This is an unusually appropriate time to mention Dr. Dight because this year is the centennial of his birthdate, July 6, 1856.

Due to the accident of birth, Dr. Dight partook of the culture of the middle of the nineteenth century, and had the crusading fire and zeal of the intellectuals of that time. The questions of those days included the abolition of slavery, Utopian community experiments, the formation of new political parties, temperance, and many social and economic reforms. Over the door of his famous "bird house" residence which was built in a tree near Minnehaha Falls, hung the motto, "Truth shall Triumph; Justice shall be Law", a quotation from a sermon on the slavery question delivered in July 1854 by Theodore Parker in Boston. This nineteenth century devotion to progress was thrown into the attack on twentieth century problems. One of these later crusades was to become the all absorbing one for Dr. Dight. This was the Eugenics Movement.

Dr. Dight received his medical degree from the University of Michigan in 1879. He then served as public health officer of Holton, Michigan. Following this there was a long period of travel in the Near East and Europe. In 1899 he joined the faculty of the Hamline University Medical College and remained on it until Hamline's medical department was amalgamated with the University of Minnesota Medical School. He remained on the staff of the latter institution for six years. Dr. Dight was the first medical director for the Ministers Life and Casualty Union.

Despite the fact that Dr. Dight's salary probably never exceeded $1500 until he was over 70 years old, his frugal living and wise investments created an estate of some $200,000 which he left to the University of Minnesota. The bequest was to be held in trust, the income to support a center for eugenics. He never paid any income taxes. How times have changed!

It is highly unlikely that the Minnesota Human Genetics League would ever have

been formed were it not for the bequest of Dr. Charles Dight to the University of Minnesota and the stipulation in his will that a Society for the promotion of the science of human genetics be established. The developments which led to such a unique bequest, and stipulation, make an amazing story.

In 1893 the following circular was distributed: "Your attention is called to the offer made by Dr. C. F. Dight to render medical services by the year. You know beforehand what your Doctor's bill for the year is. You know that in case of accident, or long continued illness from any cause, you will have all the medical attention you need without a Doctor's bill accumulating. You know that your Doctor will not prolong your case from any mercenary motive." Payments were to be made quarterly and ranged from $10 per year for a single person to $25 for a family of four. The world did *not* flock to his door to accept the offer. None-the-less, his basic idea of an annual fee for medical care is also the basic idea of Blue Shield and Blue Cross.

The urge to educate the public in matters of health and reproduction resulted in an interesting venture for Dr. Dight during the summer of 1901. He spent that summer on the Chautauqua circuit lecturing on those topics.

Dr. Dight served from 1914–1918 on the Minneapolis City Council as a *Socialist* alderman from a Republican ward. He won because he showed that both the Republican and Democratic aldermen were dragging their feet on public health matters. He forced a milk pasteurization ordinance through the Council in 1915 over determined opposition from the milk companies. He was instrumental in cleaning up the garbage collection situation and in establishing the present Public Market. Dr. Dight was a liberal in the best sense, and had little use for the many irresponsible members of the Socialist party, from which he withdrew in 1918.

By 1922 Dr. Dight had decided to dedicate his fortune to the cause of eugenics. He was a real pioneer in the field. When he initiated the eugenics movement in Minnesota the national and international movements were still in an exploratory stage. Dr. Dight was a part of those organizations. Apparently in 1922 he obtained the help of the Dean of the Medical School, E. P. Lyon, and Professor Nachtrieb, head of the Department of Zoology at the University of Minnesota, in forming the Minnesota Eugenics Society which was incorporated in 1925.

The first and only president of the Minnesota Eugenics Society was Charles F. Dight. In 1927 a peak membership of 77 persons would seem to have been a sound basis for continued growth. In this same year the American Eugenics Society published a list of eugenics lecturers for each state; Dr. Dight and Professor Dwight Minnich were the two Minnesota men listed. Indeed, Dr. Dight had a nation-wide reputation in the eugenics field. Yet by 1930 the Minnesota Eugenics Society had ceased to be active and had faded from the scene.

Why did this occur? The most important reason was probably that to the public the program did not seem urgent, and the early hopes for an immediate improvement of mankind by simple devices such as sterilization, had foundered. Another reason was that few prominent laymen had been recruited. No social reform will succeed without the eventual backing of some of those who hold the social power. Finally, if an organization is to grow, there must be a paid secretary to handle the details and maintain a tidy administration.

The death of the Minnesota Eugenics Society did not mean that Dr. Dight and his associates had lost heart. It merely meant that a preliminary experiment, for which there had been no model, was completed. A decade later the spirit was to rise again from Dr. Dight's remains, and the Society was to be resurrected.

The Minnesota Eugenics Society existed between 1922 and 1930. It was at its peak from 1925 to 1927 and it was in 1927 that Dr. Dight corresponded with President Lotus D. Coffman of the University of Minnesota concerning the possible Institute of Eugenics which Dight envisaged at the University. Dr. Dight wrote his will that year and bequeathed his estate to the University for that purpose. It is rather startling that none of his other multitudinous interests, such as better training for nurses and other public health projects, were provided with even token bequests!

The provisions for the eventual utilization of his estate by the University were clearly written and at the same time general enough in nature so that they would be adaptable to a changing environment. In return for the bequest the University was to provide academic instruction in human genetics, carry on research in the field, provide a free counseling center for the public where the people could obtain answers to their questions about heredity, and finally to establish a new Eugenics Society. It was no doubt apparent to the University that Dr. Dight's estate was inadequate for the support of such an ambitious program. However, there was sufficient enthusiasm for Dr. Dight's unique concepts so that when the bequest eventually materialized it was accepted by the University.

Dr. Dight did not decrease his personal activity in the field of eugenics even though the eventual disposition of his estate had been provided for, and his Eugenics Society was defunct. Between 1921 and 1935 over 300 of his letters on eugenics were published in the Minneapolis daily newspapers. In 1927–28 he gave a series of eleven talks over station WRHM and in 1933 a series of twelve lectures over the same station. In 1935 the St. Paul Dispatch reported on an address which Dr. Dight gave before a group of 250 people. He was then 79 years old.

Everyone must die or social problems would develop which would seem insoluble even to Dr. Dight. His demise came on June 20, 1938, when he was 82 years of age.

A review of Dr. Dight's accomplishments shows a man intellectually in advance of his times. He was a radical—one who wants to try something new and is sure that it will work while most others doubt it. His idea of prepaid medical care is now acceptable to almost everyone. Few who have heard of typhoid fever or brucellosis would oppose his crusade for compulsory pasteurization of milk used by the public. There are still many who would oppose his crusade for voluntary sterilization of the unfit, and for the fit whose genetics indicate a serious likelihood of the production of further abnormal children. The opponents of voluntary sterilization are more strongly entrenched than were the milk companies that opposed pasteurization but no group can hold back permanently the benefits medicine can bestow.

It would not be fair to judge Dr. Dight merely by the amount of legislation which he inspired, significant though it was. The useful endowment which one leaves at his death is the only thing of significance. The endowment may be a collection of bird prints, good heredity in your offspring, teachings worthy of reproduction, or the few dollars and the small library left by John Harvard. Cash without ideas is not enough—but it is very useful in almost any situation. Dr. Dight left some very large ideas and a little of the where-with-all needed to activate them. Dr. Dight's basic concept that man is susceptible to genetic improvement will not die.

By authorization of the Board of Regents of the University of Minnesota, the Charles Fremont Dight Institute for the Promotion of Human Genetics began to function on July 1, 1941, under the supervision of a director, Professor C. P. Oliver, and an advisory committee. Theodore C. Blegen, Dean of the Graduate School, has

served as chairman of the Advisory Committee during the fifteen years of its existence. Two other "charter members" of the committee are still serving faithfully, Dwight E. Minnich, Chairman of the Department of Zoology, and Donald G. Paterson, Professor of Psychology.

Under the vigorous direction of Professor C. P. Oliver the program of teaching, research and public counseling and information was initiated promptly. The first bulletin of the Dight Institute contained a report by the director on the organization and aims of the Dight Institute and a biographical sketch of Dr. Dight by Evadene B. Swanson. This biographical gem has provided the facts about Dr. Dight which the present writer has repeated and reinterpreted.

It was not until 1944 in Bulletin number three of the Dight Institute, that the following sentence appears, "The subcommittee on eugenics societies has been corresponding with various American eugenics societies and is preparing plans which we hope will interest a group in organizing a eugenics society in Minnesota." Outside assistance failed to materialize and it became clear that the precise type of organization which Dr. Dight had envisaged was not the answer to the need. The problems were not as simple, nor the solutions as easy, as Dr. Dight had thought. Attempts to provide legislation regulating the reproduction of the public were not likely to succeed until more research had been done both on methods for controlling reproduction and in the basic science of human genetics. Consequently, the immediate need was for basic research in human genetics and interpretation of the results to the medical profession and the general public. Obviously the first person who needs to be informed of discoveries from related fields concerning reproduction and health is the physician. From him the patient, meaning the public, will receive eventually the proper instructions and information about genetic problems.

It was decided that a modern "research oriented" society should be established without ties with any other group. Public education would be devoted to the dissemination of knowledge concerning human genetics, eugenics, and population problems.

The organization was to be called the Minnesota Human Genetics League. The articles of incorporation were filed on October 26, 1945. The president was Dwight E. Minnich, the treasurer, Theodore C. Blegen, and the secretary, Clarence P. Oliver.

This time there was to be no lack of participation by the prominent citizens generally to be found working for all good causes. Professor Minnich approached the late Miss Helen Bunn of St. Paul, Mrs. Dorothy Atkinson and Mrs. John Cowles, Sr., of Minneapolis, with the request that they become Patrons of the League. They not only agreed to do so but gave generously of their time and energy in the work of the League over the years. Miss Bunn left a gift of $10,000 to the Dight Institute of the University as one of her three bequests for the public good. Physicians and other prominent citizens joined the new organization and have given it steadfast support during its decade of growth.

No provision was made for a paid secretary but a partial solution was found in the willingness of the director of the Dight Institute to accept this burdensome job. The new Society was launched, and it would seem that the pitfalls which led to the disintegration of the old one had been avoided, in part.

The Minnesota Human Genetics League held it first annual meeting on November 14, 1945. It is remarkable that there were forty-seven charter members and that, as far as is known, none of them was a hold-over from the old Minnesota Eugenics

Society. Apparently Dr. Minnich, the president of the new society, was not a member of the old one. Although the Dight Institute and its Advisory Committee helped organize the Minnesota Human Genetics League, the two organizations are legally entirely unrelated. The University of Minnesota had thus fulfilled the fourth and final requirement of the Dight bequest by establishing the League. The University was not obligated to guarantee the survival of the League and it was anticipated that the five officers, who were all University professors, would be replaced as the years passed by persons outside the University. The secretary is the only University of Minnesota staff member among the five officers serving in 1956.

One of the functions of the League is to augment and encourage the activities of the Dight Institute and to interpret human genetics to the physician and the public. Thus while the two organizations are legally independent their programs are functionally parallel.

In 1946 the League suffered a crisis due to the departure of its secretary, Professor C. P. Oliver. At its third annual meeting in 1947 there were only fifty-six members in good standing. It is the secretary who rounds up the new members and prevents the old ones from falling away, once the organization has been established. In 1947 a new director for the Dight Institute and secretary of the League, was found. The new man, the author of this article, like his predecessor, could not devote much time to membership recruitment but gradually the League grew so that there are now about 150 members.

The program of the Minnesota Human Genetics League is concerned with the qualitative and quantitative aspects of the population. Thoughtful people throughout the world are now accepting the related problems of the genetics and the numbers of peoples as the most fundamental ones that there are. It was not always so. The fact that many people are now seeing human biology as the crux of social changes comes through education by the press, radio and television. These distribution mechanisms obtain their data from conferences, research papers and interpretative material produced by organizations like the League.

In 1948 the League helped to sponsor a population conference on the University of Minnesota Campus. At that time it was necessary to show the public that there *was* a world population problem. The League is co-sponsoring a second population conference in late January, 1957, which has the ambitious goal of trying to define what an optimum population would be. Roughly how much food, clothing and shelter, leisure time and community resources, are needed per person to ensure a healthy progressive society?

A study initiated by the Dight Institute and the League on Huntington's chorea has led to unexpected successes. The project was started in a very modest way but attracted the attention and assistance of Dr. John S. Pearson, head of psychological services of the Department of Public Welfare of the State of Minnesota. When Dr. Pearson went to the Rochester State Hospital as the research psychologist, he interested the Superintendent and others there in the organization of a Genetics Research Program. The work started with the help of the Dight Institute and was expanded with the aid of a generous grant from the Hill Family Foundation. The program was centered around the Huntington's chorea work, now to be done on a state wide basis. A census of all choreics and their close relatives has been completed. A popular interpretive pamphlet about the disease written by Dr. Pearson is being used throughout the nation. The League financed the printing of the first 5000 copies of the pamphlet. Efforts to distinguish which half of the progeny of a choreic will later develop

the disease are being continued. Movies of patients before and after treatment with reserpine show a remarkable suppression of symptoms, which lasts for at least a few months. The understanding of the mechanism of the inheritance of the gene for the disease which the relatives and early-stage patients have obtained has resulted in voluntary and unsolicited guarantees from some that they will refrain from reproduction and further spread of the dominant gene responsible for this horrible disease. Even a few results of this sort are of great significance to the families involved, to the state and the country. A similar program is now flourishing in Michigan, and will no doubt be initiated in other states.

The research project which the League initiated and for which it assumes responsibility is a gigantic follow-up of a study made of the mentally retarded patients at the Faribault School and Colony. The patients there between 1911–1918 and their relatives were studied with geneological, psychological, and sociological techniques. The follow-up of the descendents of the patients and relatives using the same and newer methods has provided vast amounts of data. In many cases the family group of relatives of the patient comprises over 500 individuals. Every one of the 300 family groups selected for study is different in its display of mental retardation. In those families where mental retardation continues down through the generations there is an opportunity to make a future intensive study, using biochemical and other medical adjuncts, with the expectation that new diagnostic techniques, and perhaps some treatments, may be discovered which will lighten the load of the mentally retarded and their relatives in the future. Dr. Elizabeth Reed has been employed by the League to carry out this follow-up study of the families of the mentally retarded since its inception.

In addition to its research program the League has contributed to general education by bringing world authorities to Minnesota where by public addresses, conferences, and radio broadcasts an awareness and some understanding of human genetics and population problems have been engendered. Dean Laurence H. Snyder, President-elect of the American Association for the Advancement of Science, Lady Rama Rau of India, Philip Levine, M.D., Edmund Farris, M.D., and many others have left their imprint upon the people of Minnesota. This would not have happened without the League.

Thus as the Minnesota Human Genetics League completes its first decade of life it can look forward to a highly challenging program befitting its greater maturity. What has been done is a very small beginning. The opportunity ahead is practically limitless in its potentialities for human good.

Let me add a postscript by suggesting some of the circumstances under which it is feasible to establish a local eugenics society. There are interested and influential laymen in every large community who will provide their support for the organization. Only a few need be found at first, as the charter members will recruit their friends—if an interesting program is drawn up. Your most important function as a geneticist is to think of projects which will advance the science of human genetics and at the same time contribute to social progress in the not too distant future.

There is a lot of work for the geneticist in carrying out the details of arranging for public lectures, recruiting members and in the general catalysis of the society. Such is his fate. However, in return for his trouble, the society does many things for him and his science.

1) The personal contacts with the members are stimulating and gratifying.

2) The financial support of the society for a research project augments the usually meagre research funds available to him.

3) It keeps the geneticist conscious of the fact that he has an obligation to the community which feeds him. A scientist working at an endowed institution is still dependent upon the public, the endowment merely acting as a buffer between them. The endowment is under the indirect control of the community.

4) By recruiting as officers and directors, the heads of medical, university and governmental departments a large amount of useful advice and even free assistance for genetics research projects becomes available.

5) Through its members the local eugenics society is an important and effective way of bringing the new science of human genetics before responsible officials in the state government. The separate governments of the forty-eight states still produce all the laws which involve genetics and reproduction. These governments still have to learn that genetic diseases exist and that the difficult task of controlling them has just begun. The local eugenics society can make faster progress in this educational endeavor than can the individual geneticist.

6) The local eugenics society can easily justify its existence if it does nothing more than bring the science of human genetics before the public by the mass communications media readily available. By directing the scientific output of the local eugenics society the geneticist can repay in part his obligations to the public which directly or indirectly supports him.

The Estimation of the Size of Isolates Based on Census Data[1]

O. FROTA-PESSOA

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ONE OF THE PARAMETERS used by Dahlberg (1929, 1948) for the estimation of the size of isolates, n, is b, the average number of children who become adult and marry per marriage. The value of b does not need to be determined in the case of reasonably stable populations, where it can be assumed to be 2 *a priori*. This is a practical advantage. However, the use of b imposes on the formulae an inaccuracy that leads to a net lowering of the estimates. Of course Dahlberg was aware of this fact, but he did not correct the formulae, since the approximation was good enough for his purposes at the time. He says (1948, pp. 92–93):

"We have assumed that the size of the family [b] is constant, or varies only slightly. If the variation is more considerable, this must mean we have to expect a higher frequency of marriages between cousins; this in turn means that the figures given in the table [for sizes of isolates] are, throughout, rather too low. However, it would hardly be of any interest to make more correct calculations, since we are only concerned with getting an idea of the size of the isolates."

The size of the error, however, increases with b. It is desirable, then, to use corrected formulae for n when dealing with populations in the process of growth, for which b is greater than 2. These formulae can be obtained by using, instead of b, some other parameters which can be derived from the data usually collected in the censuses.

In this paper the corrected formulae will be derived and applied to the case of some Brazilian populations. This will illustrate the method and give an idea, in a particular case, of the magnitude of the error produced by the use of b.

Dahlberg's formulae can be written as below, where n_1, n_2, n_3, n_4 are the sizes of the isolates for the relative frequencies f_1, f_2, f_3, f_4 of marriages between uncle-niece and aunt-nephew, first cousins, first cousins once removed, and second cousins, respectively.

$$n_1 = \frac{4b(b-1)}{(2+b)f_1} \qquad \text{I}$$

$$n_2 = \frac{2b(b-1)}{f_2} \qquad \text{II}$$

$$n_3 = \frac{8b^2(b-1)}{(2+b)f_3} \qquad \text{III}$$

$$n_4 = \frac{4b^2(b-1)}{f_4} \qquad \text{IV}$$

[1] Paper number 18 from the Centro de Pesquisa de Genética, Faculdade de Filosofia, Universidade do Brasil. Address: Avenida Antonio Carlos 40. Rio de Janeiro, Brazil.

Received June 27, 1956.

Calling n the size of the isolates for the frequency f of marriages between any kind of cousins, Dahlberg's general formulae can be written as follows.

For marriages between cousins of *i*th degree:

$$n = \frac{2^i b^i (b - 1)}{f} \qquad \text{V}$$

For marriages between cousins once removed of *i*th degree:

$$n = \frac{2^{i+2}\, b^{i+1} (b - 1)}{(2 + b) f} \qquad \text{VI}$$

THE MARRIED AND THE FERTILE UNCLES AND AUNTS

Before analyzing the main inaccuracy in the formulae I to VI it is convenient to consider a minor one, the correction of which is worthwhile because it leads to formulae better fitted for the application of census data.

A sibship with b married[2] persons may be considered an unbiased sample of the married people in the population as far as the proportion of fertile to sterile persons among them is concerned. In other words, if F is the frequency of fertile persons among those that marry in the population, the expected frequency of fertile persons among the b individuals forming the average-sized sibship is also F. Now, in formula II, for instance, $2(b - 1)$ stands for the average number of married uncles and aunts per married person in the population. The average number of their married children, i.e., the average number of married cousins per married person in the population, is obtained by multiplying $2(b - 1)$ by b. However, the $2(b - 1)$ individuals form a biased sample of the population with respect to fertility. The value $2(b - 1)$ is obtained by excluding from a set of 2b married persons, which forms an unbiased sample, two individuals who are fertile *a fortiori*. In fact, they are excluded as being the parents of the persons for which the average number of cousins is being calculated. Thus, the frequency of fertile people among the $2(b - 1)$ persons is less than F, and the average number of married children for the $2(b - 1)$ persons is less than b. This bias tends to make the value $2b(b - 1)$ an overestimate of the average number of married cousins per married person in the population. On the other hand, the main inaccuracy to be examined in the next section introduces a greater error in the opposite direction, so that $2b(b - 1)$ is actually an underestimate. If it is assumed that $F = 1$, i.e., that every married person in the population is fertile, the bias disappears. This simplifying assumption is implied in formulae I to VI.

THE AVERAGE NUMBER OF UNCLES AND AUNTS

Let us adopt, for the moment, this assumption in order to examine the effect of the main inaccuracy alone. Let $q_1, q_2, q_3 \cdots q_k$ be the relative frequencies in the population of the sibships composed of $r = 1$, $r = 2$, $r = 3$, $r = k$ fertile persons. The average number of fertile persons per sibship is $\bar{r} = \Sigma_1^k q_r r$. Let s be the average

[2] All individuals here considered are assumed to have completed their entire lifetimes. Thus, a person who marries in any occasion is called a married person. A fertile person is one who has one or more children at any time. All sibships are considered to be completed, and strict monogamy is assumed. It is also assumed that there is no heritability or family correlations in sibships size.

number of married children per fertile person. There are, then, in the population, $q_r rs$ married children with one parent belonging to a sibship of r fertile persons. Each among these $q_r rs$ children has, one one side of the family, $r - 1$ fertile uncles and aunts. All the $q_r rs$ children have together $q_r rs(r - 1)$ fertile uncles and aunts on one side of the family. The summation of this expression gives the number of fertile uncles and aunts, on one side of the family, for all the $\Sigma_1^k q_r rs = \bar{r}s$ married children in the population. The average number of fertile uncles and aunts on one side of the family is, then:

$$\frac{\Sigma_1^k q_r rs(r - 1)}{\bar{r}s} = \frac{\Sigma_1^k q_r(r - 1)}{\bar{r}}$$

And on both sides of the family it is:

$$\frac{2\Sigma_1^k q_r r(r - 1)}{\bar{r}} = 2\left(\frac{\Sigma_1^k q_r r^2}{\bar{r}} - 1\right)$$

This becomes equal to $2(b - 1)$ when r takes only the value $\bar{r} = b$. This shows that formulae I to VI are also based on a second simplifying assumption, namely that all sibships in the population have the same number of fertile members.

THE CORRECTFD FORMULAE

Corrected formulae are obtained by discarding these simplifying assumptions. To avoid the first one, only the fertile individuals will be considered, and their number, N, will be estimated. After that it is easy to find n, since $n = N/F$ (Böök, 1956, p. 244)[3]. For dispensing with the second assumption, the sibships of each size will be considered separately, as was done above. A condensed derivation of the corrected formulae for N and n, using the frequency of uncle-niece and aunt-nephew marriages is given below. An expanded derivation will be found in the appendix, at the end of this article.

Panmixia and sex ratio 1 are assumed. If each fertile couple has, on the average, $\bar{r}$ fertile children, the fraction $2/(2 + \bar{r})$ of the population, N, is composed of "parents", and the remaining fraction $\bar{r}/(2 + \bar{r})$ is composed of "children". The expected frequency of marriages between "individuals", belonging to sibships of r individuals, and their sisters' children, or between "children", whose mothers belong to sibships of r individuals, and their mothers' sibs, is:

$$\frac{2}{2 + \bar{r}} \times \frac{q_r r}{\bar{r}} \times \frac{\bar{r}(r - 1)}{2N} + \frac{\bar{r}}{2 + \bar{r}} \times \frac{q_r r}{\bar{r}} \times \frac{r - 1}{N} = \frac{2q_r r(r - 1)}{(2 + \bar{r})N}$$

Generalizing for sibships of any number of individuals, this formula becomes:

$$\frac{2\Sigma_1^k q_r r(r - 1)}{(2 + \bar{r})N}$$

[3] The author wishes to thank Dr. James F. Crow for calling attention to the fact that the number of fertile individuals, called N in this paper, can be equated to Wright's effective population size, only if a number of conditions are met, an important one being that the number of progeny per parent has a Poisson distribution. In human populations the variance is usually about 1.5 times the mean, so the effective size is considerably less than the actual number of fertile individuals (cf. C. C. Li, 1954, *Population Genetics*, p. 321; Crow & Morton, 1955, *Evolution* 9: 202–214).

This is, then, the expected frequency for uncle-niece and aunt-nephew marriages in which the spouses are related through a female. The expected frequency for the case in which they are related through a male is, of course, the same. Thus, the total expected frequency of uncle-niece and aunt-nephew marriages in the population is:

$$\frac{4\Sigma_1^k q_r r(r-1)}{(2+\bar{f})N}$$

Therefore, the corrected formulae, based on the frequency f_1 of uncle-niece and aunt-nephew marriages, are:

$$N_1 = \frac{4\Sigma_1^k q_r r(r-1)}{(2+\bar{f})f_1} \quad \text{and} \quad n_1 = \frac{4\Sigma_1^k q_r r(r-1)}{(2+\bar{f})f_1 F} \qquad \text{VII, VIII}$$

The other formulae for N, derived in a similar manner, follow.
For first cousins marriages:

$$N_2 = \frac{2\Sigma_1^k q_r r(r-1)}{f_2} \qquad \text{IX}$$

For marriages between first cousins once removed:

$$N_3 = \frac{8\bar{f}\Sigma_1^k q_r r(r-1)}{(2+\bar{f})f_3} \qquad \text{X}$$

The general formula, corresponding to V, for marriages between cousins of *i*th degree becomes:

$$N = \frac{2(2\bar{f})^{i-1}\Sigma_1^k q_r r(r-1)}{f} \qquad \text{XI}$$

The general formula, corresponding to VI, for marriages between cousins once removed of *i*th degree becomes:

$$N = \frac{4(2\bar{f})^{i}\Sigma_1^k q_r r(r-1)}{(2+\bar{f})f} \qquad \text{XII}$$

The corresponding sizes of the isolates, n, are obtained by dividing expressions IX to XII by F.

APPLICATION TO THE CASE OF BRAZILIAN POPULATIONS

These formulae turn out to be convenient for practical use because the parameters involved, with the exception of f, can be calculated from census data.

In a series of papers by Mortara (1944) the data from the Brazilian census of 1940 have been elaborated in a way that greatly facilitates obtaining the values to be used in the formulae. He calculates, for each of twelve States of Brazil, the reproduction rate R (Boeckh's coefficient), which is a measure of the ratio of the number of women in the population to the number of daughters they produce during their entire lifetimes. The evaluation of R involves the application of the set of fertility and mortality rates, prevailing in a given period for a given population, to a hypothetical group of newly born female infants, and the computation of the number of children

they would have during their whole lifetimes (for details of the method, see Mortara 1944, 1953, 1955). For a sex ratio of 1, the average number of children born alive per woman among females born alive is 2 R; for the sex ratio of 1.05 males to each female, which prevails in the Brazilian populations, it is 2.05 R. Assuming equal rates of marriage and fertility for two successive generations, 2.05 R is also the average number, b, of married children per married woman, and the average number, $\bar{r}$, of fertile children per fertile woman.

Mortara (1944) presents tables for the frequencies p_1 , p_2 , $p_3 \cdots p_m$ of the women having $i = 1, i = 2, i = 3 \cdots i = m$ children born alive during their whole lifetimes. Let the average number of children born alive per woman among females born alive be $\bar{i} = \Sigma_1^m p_i i$. From Mortara's data it is also easy to calculate the fraction, d, of fertile women among the women born alive in the population. Assuming equal average mortality and fertility rates for members of sibships of all sizes, $r = di$, and $q_{r=di} = p_i$.

Substituting di for r and p_i for q_r in formulae VII to XII, the values obtained from Mortara's data can be used. Formula IX, for instance, becomes:

$$N_2 = \frac{2d(d\,\Sigma_1^m p_i\, i^2 - \bar{i})}{f_2} \qquad \text{XIII}$$

The values of F, needed to evaluate n knowning N or vice-versa, can also be derived from census data, but they have not been calculated for the Brazilian populations. F is always slightly smaller than 1. In order to make possible a comparison between evaluations of n based on Dahlberg's formulae and on the corrected ones, we shall assume $F = 1$, thereby making the value of N_2 in formula XIII correspond to n_2 in formula II.

Table 1 summarizes some calculations made to estimate, for a concrete case, the discrepancy between the values of n_2 obtained by the use of Dahlberg's formula II and the corresponding corrected formula XIII. The same raw data were used in both cases. The frequencies of first cousin marriages, f_2 , appearing in Table 1, were kindly communicated to us by Dr. N. Freire-Maia, from unpublished data which he computed from church records. The remaining variables were taken from data by Mortara (1944), or computed from them.

By inspection of the last two columns in Table 1, it is seen that, for Pernambuco and Alagoas, the value of n_2 calculated according to Dahlberg's formula falls short of the value calculated according to the corresponding corrected formula by more than one third. For Paraná the relative difference is a little smaller. The discrepancy between the two sets of values of n_2 is actually greater than is shown in Table 1, since F is, in reality, smaller than 1.

Freire-Maia (1952), assuming b to be close to 4, tentatively evaluated the mean size of the Brazilian isolates to be 500. This is in contrast to the value 1600 derived by Dahlberg for European isolates, where b was considered to be 2. Both calculations were made according to Dahlberg's formulae. An inspection of formulae II and IX shows that, for the usual frequency distributions of r, the greater b, the greater the error. So, it affects the estimation of the size of the isolates for the Brazilian populations more than that for the European populations, and tends to exaggerate the

TABLE 1. MEAN SIZES OF ISOLATES, n_2, CALCULATED ACCORDING TO DAHLBERG'S FORMULA II AND THE CORRECTED FORMULA XIII (ASSUMING F = 1) FOR THREE BRAZILIAN STATES

State	b	i	d	$\sum p_i i^2$	f_2	n_2	
						Form. II	Form. XIII
Pernambuco	3.97	7.23	.55	72.65	.040	590	900
Alagoas	3.88	6.91	.56	67.02	.035	639	980
Paraná	4.16	6.74	.62	59.68	.010	2629	3752

difference between the two estimates. A recalculation of the mean sizes of the Brazilian and European isolates according with the corrected formulae would permit a more reliable comparison between the population structures in Brazil and in Europe.

APPENDIX: EXPANDED DERIVATION OF FORMULAE VII AND VIII

The derivation will be made through several steps. Panmixis and sex ratio 1 are assumed.

a) If each fertile couple has, on the average, $\bar{f}$ fertile children, the fraction $2/(2 + \bar{f})$ of the population, N, is composed of "parents", and the remaining fraction $\bar{f}/(2 + \bar{f})$ is composed of "children". Let us first calculate the probability that a person, belonging to the class of "parents" and to a sibship of r individuals, marries a niece (or a nephew) who is a child of one of his (or her) *sisters*. Each person belonging to a sibship of r individuals has $\bar{f}(r - 1)/2$ nephews and the same number of nieces, on the average. Then, among the N/2 females a man belonging to a sibship of r individuals could marry in the population, $\bar{f}(r - 1)/2$ are his nieces. The probability for a given man, belonging to a sibship of r individuals, to marry a niece is, therefore,

$$\frac{\bar{f}(r - 1)/2}{N/2} = \frac{\bar{f}(r - 1)}{N}$$

The probability for this man to marry a niece who is a daughter of one of his *sisters* is half of that, or $\bar{f}(r - 1)/2N$, on the average. The probability for a woman, who is a member of a sibship of r individuals, to marry a nephew who is a son of one of her sisters is, of course, the same. Then, a *given* person, belonging to a sibship of r individuals, has a probability of $\bar{f}(r - 1)/2N$ for marrying a niece (or nephew) who is a child of one of his (or her) sisters. Now, we are interested in the probability for such a marriage involving, not a *given* person, but *any* "parent" belonging to a sibship of r individuals. This probability depends on the relative frequency, in the population, of the "parents" belonging to sibships of r individuals. This frequency is:

$$\frac{2}{2 + \bar{f}} \times \frac{q_r r}{\bar{f}};$$

and the probability for a "parent", belonging to a sibship of r individuals, to marry a niece (or nephew) who is a child of one of his (or her) sisters is

$$\frac{2}{2 + \bar{f}} \times \frac{q_r r}{\bar{f}} \times \frac{\bar{f}(r - 1)}{2N} = \frac{q_r r(r - 1)}{(2 + \bar{f})N}$$

In other words, this is the expected frequency of marriages between "parents" belonging to sibships of r individuals and their sisters' children.

b) Now let us calculate the probability that a person, belonging to the class of "children", and whose *mother* belongs to a sibship of r individuals, marries a maternal uncle (or aunt). The argument runs in a manner similar to that in section a, and we will reach the conclusion that this probability is equal to that calculated at the end of section a.

Each person whose mother belongs to a sibship of r individuals has, on the mother's side of the family, $(r - 1)/2$ uncles, and the same number of aunts, on the average. Then a man whose mother belongs to a sibship of r individuals could marry, in the population, $N/2$ females, among which $(r - 1)/2$ are his maternal aunts. The probability of a *given* man of this type marrying a maternal aunt is, therefore,

$$\frac{(r - 1)/2}{N/2} = \frac{r - 1}{N}$$

The probability of a given woman, whose mother belongs to a sibship of r individuals, marrying one of her maternal uncles is, of course, the same. Then, a *given* person, whose mother belongs to a sibship of r individuals, has a probability of $(r - 1)/N$ of marrying a maternal uncle or aunt. Now, we want the probability for such a marriage involving, not a *given* person, but *any* "child" whose mother belongs to a sibship of r individuals. This probability depends on the frequency in the population of the "children" whose mothers belong to sibships of r individuals. This frequency is

$$\frac{\bar{r}}{2 + \bar{r}} \times \frac{q_r r}{\bar{r}};$$

and the probability of a "child" whose mother belongs to a sibship of r individuals marrying a maternal uncle (or aunt) is

$$\frac{\bar{r}}{2 + \bar{r}} \times \frac{q_r r}{\bar{r}} \times \frac{r - 1}{N} = \frac{q_r r(r - 1)}{(2 + \bar{r})N}$$

In other words, this is the expected frequency of marriages between "children", whose mothers belong to sibships of r individuals, and the "children" 's maternal uncles or aunts.

c) Let us now calculate the expected frequency for cases a and b together, i.e., the expected frequency of marriages between "parents" belonging to sibships of r individuals and their sisters' children, *or* between "children" whose mothers belong to sibships of r individuals and their maternal uncles or aunts. Such a compound expected frequency is, of course, the sum of those calculated in sections a and b, i.e.,

$$\frac{2q_r r(r - 1)}{(2 + \bar{r})N}$$

d) Generalizing for sibships of any number of individuals, it follows that the expected frequency of marriages between "parents" belonging to sibships of all sizes

and their sisters' children, or between "children" whose mothers belong to sibships of any size and their maternal uncles or aunts is:

$$\frac{2\Sigma_1^k q_r\, r(r-1)}{(2+\bar{r})N}$$

In other words, this is the expected frequency for uncle-niece and aunt-nephew marriages in which the spouses are related through a female. The expected frequency for the case in which they are related through a male is, of course, the same. Then, the total expected frequency of uncle-niece and aunt-nephew marriages in the population is

$$\frac{4\Sigma_1^k q_r\, r(r-1)}{(2+\bar{r})N}$$

From this the corrected formulae VII and VIII follow.

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Genetic and Non-genetic Factors in the Etiology of Congenital Heart Disease: a Study of 1188 Cases

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INTRODUCTION

IN 1888, FALLOT described the anatomical defects of the heart in "blue babies;" his name has since been associated with the "ensemble of defects, manifold but constant," which characterize these infants. However, as early as 1777, a Dutch surgeon named Sandifort described the tetralogy now bearing Fallot's name (see Kuyjer, 1954). A century earlier, in 1664, the Danish physician, Nicolas Stenon, described the autopsy of a malformed embryo exhibiting a harelip with cleft palate, syndactyly, an abdominal wall schistasis, defects of the genitals, and a heart defect which Stenon described as *stenosis of the pulmonary artery, septal defect, and dextraposition of the aorta*. This description, quite possibly the first, of a congenital heart defect is of interest to us for the heart malformation here described was but one of numerous defects. We now know this to be a common finding in congenital heart disease (CHD).

In the survey of the literature to follow, we shall discuss the findings with regard to congenital heart disease under four broad headings, namely, (A) congenital heart defects with associated malformations, (B) congenital heart defects and heredity, (C) congenital heart defects and the influence of external factors on fetal development, and (D) the results of extensive surveys on congenital heart disease carried out prior to this date.

A. Congenital heart defects with associated malformations.—Malformations are frequently observed in association with heart disease. We might classify these associations as follows:

1. Syndromes of which CHD is but a part, such as the Ellis and van Crefeld, or Marfan's, or Kartagener syndrome.

2. Syndromes with which CHD is occasionally associated, such as Turner's syndrome (Tyler, et al., 1953, Vulliamy, 1953) or acrocephalosyndactyly (Owen, 1951).

3. CHD in individuals showing (a) another defect such as esophageal atresia (Aboud and Alfy, 1953), renal defect (Boland and Fitzgerald, 1952), a defect of the retinal blood vessels (Bonnet, 1954), a congenital absence of the spleen (Bush and Ainger, 1955), a megacolon (Demirağ, 1951), a diaphragmatic hernia (Lowys, et al., 1955), or a diaphragmatic aplasia (Cortese and Bettolo, 1955), or (b) many malformations (Bruyne, et al., 1952; Wolf, 1955).

4. CHD, usually an atrioventricularis communis, in association with mongolism. Granata, et al., (1952) observed a congenital heart defect in 24 out of 56 mongolians, and Evans (1950) reported CHD in 28 of 63 mongolians who came to autopsy before the age of five years.

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B. Congenital heart defects and heredity.—Multiple cases of CHD in a single family have been reported by numerous authors. A familial concentration, however, is not necessarily evidence of an hereditary etiology. Let us, nonetheless, summarize the conclusions suggested by these single family cases.

Gänsslen, Lambrecht and Werner (1940) surveyed the literature prior to 1940. Sixty-eight family pedigrees were studied by these authors. They cite evidence for recessive inheritance in certain cases, in particular patent ductus arteriosus and atrial septal defect, and for possible dominant inheritance in other traits. Other authors provide evidence for an intrafamilial homogeneity of the clinical type of CHD: patent ductus arteriosus (Kjaergaard, 1946), pulmonary stenosis (Coblentz and Mathivat, 1952), interventricular septal defect (Tucker and Kinney, 1945), etc. Other families, however, show no such intrafamilial homogeneity (Stein and Barber, 1945). All of the simpler modes of inheritance save sex-linkage have been suggested by various authors.

Situs inversus and dextrocardia have frequently been reported in related individuals as well as in twins. Doolittle (1907) described a family with two cases of dextrocardia and suggested the possibility of dominant inheritance. The same author finds no evidence, from other cases of situs inversus viscera or dextrocardia, of an increase in consanguinity, but he does point out the high frequency of twinning in families in which a case of dextrocardia has been observed. Furthermore, he reports eight cases of twin pairs in which only one twin exhibited dextrocardia. Doolittle believes that these cases of discordance in twins may afford an explanation of the mechanism of dextrocardia.

Torgersen (1948) reported 12 cases of dextrocardia in MZ twins (six of which were concordant) and three cases of concordant DZ twins. He believes that the discordant MZ twins do not support the hypothesis of a mirror mechanism since extrinsic factors acting on only one of the twins could produce discordance. He further asserts that the concordant MZ twins do not necessarily support the hypothesis of a genetic origin, since extrinsic factors may well influence both twins.

Lowe and McKeown (1954) report ten cases of dextrocardia and 20 cases of situs inversus. They estimated the frequency of isolated dextrocardia to be 1 in 29,000 births and that of situs inversus 1 in 7,000. Only one of their cases of situs inversus was familial. They also report one case of MZ twins discordant with respect to situs inversus and conclude that the trait is not inherited as if due to a single recessive gene with full manifestation, thus contradicting Cockayne (1938), who asserted that situs inversus is a recessively inherited trait and that there exist different types of partial transposition.

Other publications (Reinhardt, 1912; Pezzi and Carugati, 1924; Araki, 1935; Kean, 1942; Helweg-Larsen, 1947, 1948; Jeune and Confavreux, 1948) support the hypothesis that situs inversus and dextrocardia are due to a very complex genetic mechanism, and that mirror imaging with regard to these traits is a very frequent occurrence in twins. The occurrence of situs inversus or dextrocardia in single individuals may be due to the survival of only one of two such "mirror" twins.

Finally, cases of twins exhibiting CHD other than dextrocardia and situs inversus have been described by several authors (Gänsslen, et al., 1940; Benesova and Sikl, 1954; Bertoye, et al., 1952; Graves et al., 1954; Tumay and Gurson, 1952; McClintock, 1945; Morison, 1949; Wade, 1952; Goldman and Stern, 1952). Many more cases of MZ twins (of which approximately half are concordant and half are discordant) than DZ twins are found in the literature. This fact suggests that only those

cases which support a particular point of view or are uncommon findings are published, and of course, seriously limits the usefulness of the data; nevertheless the occurrence of discordant MZ twins attests to the role of non-genetic factors in the etiology of CHD. Morison (1949) believes that at an early embryonic stage twins compete with one another for nutrition and, as a consequence, one of them may suffer from a transitory lack of nutrition, which in turn leads to the occurrence of a malformation. The malformed twin may then be carried, as it were, to delivery by the non-malformed twin through the agency of a mutual circulation. Concordance in MZ twins may occur through the influence of extrinsic or genetic factors whereas uniform concordance in DZ twins would appear to suggest primarily extrinsic factors. A case of concordant DZ twins where the concordance is probably due to extrinsic factors has been reported by Bertoye, et al. (1952). On the whole, the published twin data might seem to suggest a greater importance of extrinsic factors than the genetic factors in the etiology of CHD.

C. Congenital heart disease and external factors influencing fetal development.—During the embryonic stage, non-genetic factors may either interrupt normal growth of the heart or provoke growth in an abnormal direction. Gregg (1941) demonstrated the influence of rubella on fetal development. Others confirmed his findings (Swan, et al., 1943, 1944; Carruthers, 1945; Reese, 1944; Rones, 1944; and Albaugh, 1945). The frequency of malformation among the offspring born to mothers having rubella has been variously estimated to be from 25 per cent to about 80 per cent.

Carruthers (1945) concluded that the severity of a malformation tends to decrease the later in pregnancy the infection occurs and Conte, McCammon, and Christie (1945) showed that rubella causes malformations only if the mother is affected during the first trimester of pregnancy. From data reported by Le Lorier (1955) in a survey of the literature, it would seem that the risk of malformation is 60 to 80 per cent if rubella occurs during the first trimester. However, other observations (Krugman and Ward, 1954; Logan, 1951) suggest that the risk is more likely of the order of 20 per cent.

In addition to rubella, other viral or non-viral diseases may be etiologic factors in congenital malformations. While Buck (1955) doubts that this is so, Landtmann (1948) has reported that in 45 per cent of 73 pregnancies terminating in the birth of a malformed child the mother had had a viral or non-viral disease during pregnancy (12 cases of acute disease, 4 of tuberculosis, 1 of jaundice, etc.), while in a control group of 200 mothers only 14 per cent reported similar episodes. We have reported elsewhere (Lamy et al., 1951) a high frequency of infectious diseases during pregnancies giving rise to malformed children.

Other external factors which possibly play a role in the etiology of CHD are maternal age and birth rank (MacMahon, 1952; Landtmann, 1948), birth interval (Murphy, 1947), the altitude of the birthplace (Alzamora, 1953), and the month of birth (Rutstein, et al., 1952). The latter report avers that the frequency of patent ductus is higher during the winter months (from October to January), and this may reflect the high frequency of rubella during the early months of such pregnancies (late winter, early spring).

D. Extensive Surveys.—At the present time extensive surveys on CHD would seem to us to be one of the more fruitful approaches to the etiology of CHD.

Such surveys yield information with regard to genetic factors as well as familial incidence, non-genetic factors, and the occurrence of unusual events during pregnancy.

McKeown, MacMahon, and Parsons (1953) have reported that 1.8 per cent of the siblings of 431 propositi distributed in 425 sibships exhibited CHD. This frequency was estimated to be 0.32 per cent in the general population. The authors did not observe an increase of CHD among the parents or the first cousins of the propositi. They observed a slight increase in the consanguinity rate only in the case of dextrocardia and situs inversus.

Richards, et al., (1955) have observed a CHD frequency of 0.83 per cent in a group of 6,053 infants. This frequency was shown to vary from 7.7 per cent in stillborn children or children dying during the first month of life, to 0.6 per cent in children who had reached the second month of life. These authors also noted that older mothers more frequently produce malformed children. They believe that neither external factors affecting fetal development nor genetic factors play a major role.

Anderson (1954) found in a study of 117 cases of patent ductus arteriosus that both genetic factors and extrinsic factors are important.

Polani and Campbell (1955) concluded from a study of 377 families with one or more cases of CHD that, (1) malformations of the aorta appear more frequently in boys than in girls, (2) the frequency of CHD is twenty times higher among the siblings of the propositi than in the general population, (3) the rate of consanguineous marriages was not increased among the parents of the propositi, and (4) there was no evidence for extrinsic factors such as maternal age, birth rank, or abnormalities during pregnancy.

Finally, we have, in earlier surveys (Lamy and Schweisguth, 1947, 1948; Schweisguth, 1952) reported evidence suggesting the importance of both genetic and extrinsic factors. This survey now encompasses 1,188 cases of CHD. The present paper will be devoted to a discussion of these cases.

MATERIALS AND METHODS

The 1,188 index patients with CHD here reported were examined during the years 1946 through 1955 by Professors Robert Debré and Pierre Soulié and their assistants at the "Consultation de Cardiologie de la Clinique Médicale de l'Hopital des Enfants Malades" in Paris. The medical information available for these 1,188 cases varies somewhat as indicated in Table 1. Briefly, in 72.6 per cent diagnosis has been established on clinical grounds with (1) X-ray examination and E.K.G. (all cases), and (2) catheterization (most cases); in 14.3 per cent a surgical operation confirmed a previous clinical diagnosis; and in 13.1 per cent death followed the first consultation and necropsy was performed in 61.9 per cent of these cases.

Each of the index cases, the propositus, has been assigned to one of the following eight clinical subgroups:

Group I: 238 cases (20.03%): Fallot's tetralogy and pentalogy.
Group II: 56 cases (4.71%): Pulmonary valvular stenosis either as a unique defect or accompanied by atrial septal defect.
Group III: 136 cases (11.45%): Patent ductus arteriosus with or without another heart defect.
Group IV: 143 cases (12.04%): I.V. septal defect.
Group V: 97 cases (8.16%): Atrial septal defect.
Group VI: 54 cases (4.55%): Coarctation of the aorta.
Group VII: 332 cases (34.04%): Precise diagnosis has either not been possible or the defect was extremely complex.

Group VIII: 132 cases (11.11%): Well defined but uncommon anatomical defects. Among these are:

1. Abnormal coronary arteries: 4 cases (0.36%): 2 ♂, 2 ♀
2. Atrioventricularis communis: 15 cases (1.26%): 5 ♂,10 ♀
3. Transposition of the great vessels: 23 cases (1.94%): 17 ♂, 6 ♀
4. Eisenmenger's complex: 20 cases (1.68%): 10 ♂, 10 ♀
5. Moderate truncular pulmonary atresia: 10 cases (0.84%): 1 ♂, 9 ♀
6. Valvular aortic stenosis: 16 cases (1.35%): 14 ♂, 2 ♀
7. Tricuspid atresia: 19 cases (1.60%): 10 ♂, 9 ♀
8. Dextrocardia: 13 cases (1.09%): 6 ♂, 7 ♀
9. Situs inversus with or without congenital heart disease: 12 cases (1.01%): 4 ♂, 8 ♀

The relative frequencies observed in this survey are certainly a biased estimate of the true frequencies at birth. In particular the frequencies of those types of CHD which cause death at a very early age are underestimated. This is of course inherent in the method of ascertainment and could only be overcome by a prospective study. Likewise, a prospective study based solely on surgical or post-mortem diagnosis would be the only study giving entire confidence in the diagnosis. But such a study would not encompass cases of CHD which do not lead to the operating or autopsy tables. In order to approach such ideal conditions we have classified each case in a particular group only on very firm diagnostic grounds, and cases which appeared slightly dubious were classified in group VII.

The following information was obtained for each of the 1,188 cases:

1. The propositus:
   a. Age at first consultation
   b. Sex
   c. Social status of parents
   d. Birthweight
   e. Diagnosis (clinical, surgical, post-mortem)
   f. Associated malformations
2. Propositus' parents:
   a. Consanguinity
   b. Mother's and father's ages
   c. Mother's and father's histories
   d. Information on mother's pregnancy
3. Propositus' siblings:
   a. Number of males, females, miscarriages, stillbirths, twins
   b. Congenital malformations
   c. Birth rank of propositus
4. Other relatives of the propositus:
   a. Number and relationship of malformed persons, if any

The above information was obtained either by direct questioning of the parents of the index cases, or by use of the patient's record in association with a supplementary mail questionnaire. When siblings of the propositi were reported to exhibit a CHD or any other defect the diagnosis was confirmed by clinical examination. In the case of more distant relatives a report was obtained from the attending physician.

Comparable information was obtained on a control group of 660 individuals randomly selected from the general population. To minimize possible extraneous differences between the two groups, special attention was directed toward ensuring comparable social and familial backgrounds, age of index cases, place of origin, etc. It should be pointed out, however, that the control group was drawn primarily from Paris, whereas the study group was drawn from all over France. This could adversely affect the consanguinity rate, but as we shall see, the rate observed in the control group compares favorably with that generally observed in France.

THE DATA

A. Age at first consultation.—Half of the patients with congenital heart disease were examined for the first time before the age of 3 years; the mean age at first examination for all patients was 3.62 years.

B. Sex ratio.—There were 605 males (50.92 per cent) and 583 females (49.03 per cent) among the 1,188 cases of CHD (Table 1). These figures agree with a theoretical sex ratio of 1:1 ($X^2 = 0.20$; $P = 0.60$) and with the sex ratio observed in the control group, namely, 335 males to 325 females ($X^2 = 0.01$; $P = 0.90$).

The sex ratio is not uniform, however, in all clinical subgroups. In Group III (patent ductus arteriosus) the sex ratio deviates significantly from 1:1 ($X^2 = 13.90$; $P = 0.0002$) as well as from the control. Patent ductus appears to occur, on the average, three times as frequently among females as among males (Table 1). In Group VI (coarctation of the aorta), the frequency of affected males is higher than affected females, although this difference is not significant ($X^2 = 2.44$; $P = 0.15$), and in Group VIII, the frequency of valvular aortic stenosis is significantly higher in males ($P = 0.024$). These results will be discussed in detail later.

C. Maternal age and birth rank.—Adequate information with regard to maternal age and birth rank is available for 1,177 cases in the CHD group, and all of the cases in the control group. The mean maternal age for the CHD group, in its entirety, is lower than that for the control group, but not significantly so (28.39 years vs. 28.84 years) (Table 2). Mean birth rank, on the other hand, is significantly different between the groups, with the CHD group having a mean of 2.23 whereas the control group has a mean of 2.03 ($t = 2.92$, $P = 0.01$).

Among the eight clinical subgroups, the mean maternal ages and mean birth rank show little variation when compared with the entire CHD group.

In Table 3 are given the results of the test of the independence of maternal age and birth rank effects. When birth rank is held constant, maternal age is lower in the CHD group than in the control group (except for rank 6). This difference is significant, however, only for ranks 1 and 2 ($t = 2.24$ and 2.27, $0.01 < P < 0.05$). If maternal age is held constant, birth rank is significantly higher in the CHD group for maternal age intervals 25–29, and 30–34 ($t = 3.11$ and 2.62, $P < 0.01$).

Paternal age was accurately known for 779 of the index cases. Mean paternal age for these cases (Table 4) was 31.13 years and mean maternal age, in the same group, was 28.23 (in the entire group, mean maternal age was 28.39). In the control group, mean maternal age was 28.86 and mean paternal age was 30.80 years. The difference between the two mean paternal ages is not significant ($t = 0.84$, $P = 0.40$). Significant differences do exist, however, between the father-mother age correlation coefficients in the two groups. In the CHD group, $r = 0.75$ and in the control group $r = 0.56$ (Table 4).

D. Consanguinity.—In Table 5 are given the data concerning relationship be-

TABLE 1. DISTRIBUTION OF CONGENITAL HEART DEFECTS BY SEX, AGE AT FIRST CONSULTATION, AND TYPE OF DIAGNOSIS (CLINICAL, OPERATIVE, AUTOPSIES)

Clinical Group	Sex	Clinical dx.	Operative dx.	Deaths[1]	Total[2]
I. Fallot's tetralogy and pentalogy	♂	65	50	16(9)	131(55.04)
	♀	53	33	21(11)	107(44.96)
	Tot.	118	83	37(20)	238(20.03)
II. Pulmonary valvular stenosis	♂	18	6	5(3)	29(51.78)
	♀	16	5	6(5)	27(48.22)
	Tot.	34	11	11(8)	56(4.71)
III. Patent ductus arteriosus	♂	16	21	1(1)	38(27.94)
	♀	63	34	1(1)	98(72.06)[3]
	Tot.	79	55	2(2)	136(11.45)
IV. I.V. septal defect	♂	72	0	7(4)	79(55.24)
	♀	63	0	1(1)	64(44.76)
	Tot.	135	0	8(5)	143(12.04)
V. Atrial septal defect	♂	40	1	2(0)	43(44.33)
	♀	52	0	2(2)	54(55.67)
	Tot.	92	1	4(2)	97(8.16)
VI. Coarctation of the aorta	♂	23	9	3(1)	35(64.81)
	♀	14	3	2(1)	19(35.19)
	Tot.	37	12	5(2)	54(4.55)
VII. CHD with no precise diagnosis	♂	152	1	28(17)	181(54.52)
	♀	124	2	25(13)	151(45.48)
	Tot.	276	3	53(30)	332(34.04)
VIII. Uncommon anatomical defects	♂	45	4	20(16)	69(52.27)
	♀	47	1	15(11)	63(47.73)
	Tot.	92	5	35(27)	132(11.11)
Total CHD group	♂	431	92	82(51)	605(50.92)
		49.94	54.11	53.90(53.13)	
	♀	432	78	73(45)	583(49.08)
		50.06	45.89	47.10(46.87)	
	Tot.	863	170	155(96)	1188(100.00)
		72.64	14.31	13.05(61.94)	

[1] The figures in parentheses refer to the number of autopsies.

[2] The second figures in the "♂" and "♀" rows refer to the percentage of each sex, and they add to 100, while the second figure in the "Tot." row refers to the percentage of each clinical group with regard to the total CHD group.

[3] The sex-ratio is significantly different from 1:1 ($P < 0.01$).

tween the parents of the propositi. In each case, the coefficient of relationship, C, has been calculated, where

$$C = \sum \left(\tfrac{1}{2}\right)^n$$

and n is equal to the number of links in the ancestral chain joining the two parents and passing through a common ancestor, and summation is over all different paths connecting the parents. To obtain a total coefficient of relationship, say $\bar{C}$, for each of the eight clinical groups, as well as for the total CHD and control groups, the individual coefficients have been summed, and divided by N, the total number of matings in the particular group.

TABLE 2. MEAN MATERNAL AGE AND MEAN BIRTH RANK IN EACH CLINICAL SUBGROUP, IN THE TOTAL CHD GROUP, AND IN THE CONTROL GROUP

	I	II	III	IV	V	VI	VII	VIII	Total	Control
N	233	55	135	143	96	53	331	131	1177	660
Mean Maternal age	28.25	29.40	28.30	28.00	28.65	27.85	28.40	29.00	28.39	28.84
Standard error of the mean, $s_{\bar{x}}$	0.41	0.82	0.59	0.51	0.71	0.84	0.36	0.53	0.19	0.24
Mean birth rank	2.25	2.35	2.14	2.10	2.04	1.98	2.35	2.32	2.23	2.03
$s_{\bar{x}}$, id.	0.10	0.20	0.14	0.11	0.14	0.12	0.09	0.13	0.04	0.05

TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS OF TOTAL CHD GROUP AND CONTROL GROUP

	Mean Maternal Age	Mean Birth Rank
d (difference)	0.45	0.194
s_d	0.31	0.066
t	1.47	2.920

Two findings emerge from a comparison of the total coefficients of relationship, namely, (1) consanguinity is 3.6 times higher in the CHD group than in the control group, and (2) $\bar{C}$ varies considerably from one clinical subgroup to another. It is highest in situs inversus, very high for atrioventricularis communis and dextrocardia, and is lowest in Group I (Fallot's tetralogy).

Analysis of the 28 sibships arising from consanguineous matings showed the following:

(1) In 2 cases (7.1%) a sibling of the propositus was also suffering from CHD. In the entire group of 1,188 sibships, this frequency was only 2.5 per cent. Though the observed difference is not significant, it may be that the frequency of malformed children is increased among the offspring of related parents.

(2) In 5 cases (17.8%) the propositus exhibited a malformation in addition to CHD. In all 1,188 propositi, this frequency was also 17.8 per cent. Hence consanguinity does not appear to increase the frequency of additional malformations.

(3) In 3 cases (10.7%), a sibling of the propositus exhibited a malformation other than a heart disease whereas in the whole group of 1,188 sibships this frequency was only 0.93 per cent (20 out of 2045). The difference between these two frequencies is significant ($P < 0.05$).

(4) The frequency of all individuals (propositi excluded) exhibiting a congenital heart disease or another malformation was 17.8 per cent in consanguineous sibships, whereas this frequency was only 2.4 per cent (50/2045) in all 1,188 sibships. The difference between these two values is significant ($P < 0.05$).

Collectively, these observations suggest that consanguinity increases the frequency of all malformations, and only secondarily the frequency of congenital heart disease. It would also seem that this increase does not influence the number of malformations possessed by a single individual.

E. Infection, threats of miscarriage and premature delivery, and other abnormalities during pregnancy.—Some 232 (19.5%) of the 1,188 mothers in the CHD group have reported unusual events during the pregnancies terminating in the index cases. In the control population, only 72 (10.9%) of the 660 mothers reported similar events. These two frequencies are significantly different ($P < 0.001$), and suggest

TABLE 3. TEST OF INDEPENDENCE OF MATERNAL AGE AND BIRTH RANK EFFECTS

Birth Rank..	1		2		3		4		5		6		7		Total		CHD		Control		t
Age of Mother	CHD	Control	CHD	Control	CHD	Control	CHD	Control	CHD	Control	CHD	Control	CHD	Control	CHD	Con-trol	Mean birtn rank	$s_{\bar{x}}$	Mean birth rank	$s_{\bar{x}}$	
15–19	56	10	7	3		1	1								64	14	1.16	0.06	1.35	0.11	1.01
20–24	198	101	78	40	18	16	6	1	1		1				302	158	1.46	0.04	1.47	0.06	0.14
25–29	136	124	111	62	70	29	22	10	7	4	2	2	1		349	231	2.04	0.06	1.76	0.07	3.11
30–34	52	40	67	56	55	27	38	12	16	2	5	7	5	1	238	145	2.72	0.09	2.34	0.11	2.62
35–39	39	12	30	18	33	16	22	13	8	3	9	3	14	5	155	70	3.08	0.15	3.08	0.19	—
40–44	13	4	9	9	8	9	12	4	10	1	4	1	10		66	28	3.74	0.22	2.71	0.23	2.00
45+		3		3		1		1		2			3	4	3	14	7.00	—	3.86	0.68	—
Total	494	294	302	191	184	99	101	41	42	12	21	13	33	10	1177	660					
Mean age	25.55	26.45	28.05	29.25	30.45	31.15	32.45	33.40	34.25	34.50	35.10	33.15	38.36	40.50							
$s_{\bar{x}}$	0.27	0.29	0.31	0.43	0.38	0.42	0.55	0.85	0.86	2.09	1.16	1.08	0.83	1.65							
t	2.24		2.27		1.22		1.83		0.11		1.43		1.22								

TABLE 4. MEAN MATERNAL AGE AND MEAN PATERNAL AGE. COEFFICIENTS OF CORRELATION

	Maternal Age		Paternal Age	
	Control	CHD	Control	CHD
N	660	779	660	779
Mean age	28.86	28.23	30.80	31.13
σ	6.09	6.24	7.38	7.37
s	0.24	0.22	0.29	0.26
t			0.84	
r	0.564		0.749	

Significance test for difference of correlation coefficients t (z transformation) = 7.45 and P = 0.007

TABLE 5. THE MEAN COEFFICIENT OF RELATIONSHIP ($\bar{C}$) AMONG THE PARENTS OF THE PROPOSITI BY CLINICAL SUBGROUPS AND FOR THE CONTROL

CHD Group	N	Relationship of parents	$\bar{C} \times 10^3$
I	238	1 third cousin 1 second cousin once removed	0.098
II	56	2 first cousins	4.464
III	136	1 first cousin once removed 2 first cousins	2.298
IV	143	1 first cousin	0.874
V	97	3 first cousins	3.866
VI	54	1 second cousin	0.579
VII	332	1 brother-sister 5 first cousins 1 second cousin once removed 1 third cousin	3.459
VIII			
Situs inversus	12	1 first cousin 1 second cousin 1 first cousin once removed	18.170
Dextrocardia	13	1 first cousin	10.420
Eisenmenger's complex	20	1 first cousin 1 second cousin	7.820
I.A.V. Communication	15	1 first cousin 1 second cousin	10.420
Total	1188		2.460
Control	660	3 first cousins 2 second cousins 1 third cousin	0.6748

that abnormal symptoms during pregnancy may be twice as common in the CHD group as in the control group.

This difference could, of course, reflect a tendency for mothers giving birth to abnormal children to seize upon unusual events to account for the abnormality in their child. It seems unlikely, however, that this explanation would account for all of the difference which has been observed, since every effort was made to elicit positive histories from the control group.

1. Rubella.—Fourteen cases of rubella, of which 11 were positively diagnosed and 3 were very probably rubella, occurred during the early stages of pregnancy, 10

TABLE 6. THE FREQUENCY OF ABNORMALITIES DURING PREGNANCY BY MATERNAL AGE

(The figures in parentheses under the figures defining the age groups refer to the number of propositi in each maternal age group; the figure on the left refers to the CHD group, that on the right to the controls.)

Maternal Age Group	Rubella				Threats of miscarriage and premature delivery				Infections				Other abnormalities				Total			
	CHD		Control		CHD		Control		CHD		Control		CHD		Control		CHD		Control	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15–19	2		0		4		1		2		0		6		3		14		4	
(64) (14)		3.13				6.25		7.14		3.13				9.38		21.42		21.87		28.56
20–24	4		0		18		8		21		6		18		7		61		21	
(302) (158)		1.32				5.96		5.06		6.95		3.80		5.96		4.43		20.20		13.29
25–29	7		0		21		6		22		5		25		10		75		21	
(349) (231)		2.01				6.02		2.60		6.30		2.16		7.16		4.32		21.49		9.09
30–34	0		0		16		6		8		3		17		6		41		15	
(238) (145)						6.72		4.14		3.36		2.07		7.14		4.14		17.23		10.35
35–39	1		0		6		2		8		2		15		2		30		6	
(155) (70)		0.65				3.87		2.86		5.16		2.86		9.68		2.86		19.36		8.58
40–44	0		0		2		2		4		0		5		3		11		5	
(66) (28)						3.03		7.14		6.06				7.56		10.71		1.67		17.85
45+	0		0		0		0		0		0		0		0		0		0	
(3) (14)																				
Total.....	14		0		67		25		65		16		86		31		232		72	

during the first month, 3 during the second month, and 1 in the third month. No cases were observed after the third month, nor were any cases observed in the control group at any time.

The frequency of rubella varies considerably from one subgroup to another. In 5 of the 14 cases, the pregnancy terminated in an infant with patent ductus arteriosus although the general frequency of this defect is only 11.45 per cent.

2. Threats of miscarriage and premature delivery.—Such symptoms occurred in 67 pregnancies (5.64%) in the CHD group whereas among the control group, the comparable figure was 3.64 per cent. The difference between these two frequencies is greater when only the first month of pregnancy is taken into account—4.13 per cent in the CHD group as opposed to 1.66 per cent in the control group.

The frequency of "threats of miscarriage" varies in the different maternal age groups. They are twice as frequent among mothers under 35 years of age as among mothers over 35 years. In the control group, the frequency of "threats of miscarriage" remains constant in the different maternal age groups (Table 6). It would seem that (a) the threat of miscarriage occurs twice as frequently in pregnancies terminating in a malformed child as in pregnancies terminating in a normal child, and (b) in the study group, the threat of miscarriage is twice as common among young mothers (under 35) as among older mothers (over 35).

3. Infections.—The frequency of infection during pregnancy is twice as high in the study group as in the control group. In most cases, the diagnosis during pregnancy of a microbial or viral infection other than rubella was made by a physician at the time of the prenatal examinations required by law. Among the latter infections recorded are measles, grippe, typhoid fever, pleurisy, E. coli infection of the urinary tract, tuberculosis, etc. Sixty-five such cases were observed (5.47%) in the CHD group whereas in the control only 16 mothers (2.42%) were observed to have had infections of this variety.

In the CHD group, episodes of infection were highest during the first month of pregnancy (20 cases) and decreased steadily thereafter (15 cases in the second month, 7 cases in the third month, and 1 case which persisted throughout the first trimester). In all, 43 cases were observed during the first three months of pregnancy. In the control group, 12 of the 16 cases of infection (of which 7 were E. coli infections of the urinary tract) were recorded during the first trimester of pregnancy. By and large, the infections experienced by the CHD group during the first trimester were more serious than those in the control group. After the first three months, the two groups were comparable.

4. Other unusual events during pregnancy.—With regard to other irregularities during pregnancy, we observed 11 cases of persisting menses in the CHD group (only 1 in the control); traumas (surgical operations, falls, etc.) in the CHD group were eleven times (11 cases vs. 1) as high as in the control group during the first three months, and six times (6 cases vs. 2) higher during the last two trimesters. It is worth noting that eight cases of systemic hormonal treatment without any threat of pregnancy interruption were observed among the mothers of the CHD patients. In one case, this treatment was instituted because of a history of frequent miscarriage. One case of treatment with ultraviolet rays during the first seven months without interruption of the pregnancy was observed. Sixteen cases of incurable vomiting, 9 cases of grave emotional shock, and 13 of other unusual events were also observed among the mothers of the CHD propositi.

5. The different clinical groups.—The groups do not show the same frequencies

TABLE 7. FREQUENCY OF CHD CASES AMONG THE SIBLINGS OF THE PROPOSITI

	Clinical type of Congenital Heart Disease								
	I	II	III	IV	V	VI	VII	VIII	Total
Number of siblings	404	107	249	213	161	78	594	239	2045
Number of affected siblings	4	4	3	1	2	2	13	1	30
Per cent	1.0	3.74	1.2	0.47	1.24	2.56	2.19	0.42	1.46

of irregularities during pregnancy. The greatest frequency (25%) of irregularities was observed in Group III (patent ductus arteriosus) and as mentioned, this group has the greatest frequency of rubella. In Groups I and IV (Fallot's tetralogy, and I.V. septal defect respectively) the frequencies were almost identical (22.3 and 22.4%). Among the remaining clinical subgroups, the frequency decreases in this order: Group V (atrial septal defect), VII (unspecified heart diseases), VI (coarctation of the aorta), VIII (miscellaneous), and II (pulmonary valvular stenosis).

F. Familial incidence of congenital heart disease.—

1. In the sibship.—Table 7 presents the familial incidence of congenital heart disease for each clinical subgroup as well as for the total CHD group. Among the siblings of the propositi, 30 cases of CHD have been registered (Tables 7 & 8). In no instance does the per cent of affected individuals approach that to be expected under a hypothesis of a single, fully penetrant gene. This is hardly an unexpected finding.

While the data are not compatible with a simple genetic explanation, the incidence of CHD among the siblings of the propositi is elevated in all clinical subgroups over that of the control group. In the control group we did not observe a single instance of congenital heart disease among the 1,487 offspring distributed among the 660 sibships. Since our data do not permit a precise estimate of the frequency of CHD in the general population nor are there other estimates in the French literature, we shall accept the incidence given for England by Polani and Campbell (1955) of 1 case per thousand individuals. This rate would lead to an expected number of 1.48 affected persons in our control group. The probability of observing no cases among the 1,483 individuals in the control if the true rate is 0.001 is approximately 0.23.

Other frequency estimates of CHD in the general population have been proposed. Anderson (personal communication) believes that the true frequency is approximately 0.7 per cent. This figure would not be in agreement with the control group. However, even if it should be so, one may conclude that the frequency of congenital heart disease is elevated among the siblings of affected individuals. While this elevation is not consistent with that to be expected on a single gene hypothesis, the elevation may amount to a seven-fold increase in the disease rate if one accepts 0.7 per cent as the frequency of CHD in the general population.

2. Among the parents.—In both CHD and control groups we failed to observe a single instance of congenital heart disease among the parents of the index cases.

3. Among the first cousins of the propositi.—In the CHD group 20 cases of heart malformation have been observed (Table 8). The total number of first cousins is not sufficiently well known, however, to permit computing a frequency. In the control group, only one case, Fallot's tetralogy, has been observed. Since the ratio of propositi, to control cases is 2:1 while the ratio of secondary cases in these two groups is 20:1,

TABLE 8. CONGENITAL HEART DEFECTS IN THE RELATIVES OF THE PROPOSITI

Group	Sibship[1]		1st cousins		Description of CHD
	Same	Other	Same	Other	
I	2		2		Fallot's tetralogy
		2			Unspecified CHD
II	1				Pulmonary valvular stenosis
		1			Fallot's tetralogy
		2			Unspecified CHD
III		3			Unspecified CHD
			1		Patent ductus arteriosus
				3	Tetralogy—I.V. septal defect—unspecified
IV	1		1		I.V. septal defect
		½ sister		2	Unspecified CHD
				1	Fallot's tetralogy
V	1		1		Patent foramen ovale
		1		1	Fallot's tetralogy
VI	1				Coarctation of the aorta
		1			Fallot's tetralogy
VII		1			Aorta-pulmonary communication
		1			Patent ductus arteriosus
		1		2	Fallot's tetralogy
		½ sister			Fallot's tetralogy
		2			Fallot's tetralogy (2 brothers)
		8		5	Unspecified CHD
VIII	1				Eisenmenger's defect (as in propositus)
Total.....	7	25	5	15	
Control.....			1		Fallot's tetralogy

[1] The headings "Same" and "Other" indicate whether the clinical type of CHD observed in the relatives of the propositus was or was not of the same type as in the propositus.

it is very likely that the frequency of congenital heart disease is increased among the cousins of the index cases.

4. The clinical similarity of multiple cases of congenital heart disease within a family. —When a heart malformation occurs among the siblings, or other relatives of the propositus, it is generally not of the same clinical or anatomical type as the malformation found in the propositus. We have observed (a) only 7 cases (out of 30) where propositus and sibling possessed the same malformation, (b) 5 cases (out of 20) where the propositus and a first cousin had the same malformation, and (c) 4 cases (out of 43) where the propositus and some relative other than parent, sibling, or first cousin exhibited the same congenital malformation of the heart. On the average, then, when a relative of the propositus has a congenital heart disease the disease is of the same variety as that exhibited by the propositus in only 1 out of 4 cases. These facts suggest that there is a familial increase in the frequency of congenital heart disease but this increase is non-specific. However, it should be borne in mind that the adequacy of the diagnosis of CHD among the relatives is not on a par with that in the propositi.

TABLE 9. CONGENITAL DEFECT IN ADDITION TO CHD PRESENT IN THE PROPOSITI

Type of defect	I	II	III	IV	V	VI	VII	VIII	Total CHD Group	%	Control
Mongolism	7		2	5	5		17	16	52	24.53	
Angiomatosis and nevi	1	2		2	4	1	4		14	6.60	3
Kyphoscoliosis		1	2	2	2		6	1	14	6.60	
Psychomotor retardation	5	1	2		2		1		11	5.19	
Multiple malformations of hands and upper limbs	3		1		2	1	1	1	9	4.25	
Bony chest deformities			1	1	2		2	1	7	3.30	
Cataract and other multiple defects		1	2				4		7	3.30	
Cataract			2	1			3		6	2.83	
Harelip and cleft palate	3					1	2		6	2.83	1
Club foot	1				1		3	1	6	2.83	
Severe multiple malformations	1			1			4		6	2.83	
Defect of the alimentary canal and associated organs	1			2			2		5	2.36	
Cranial malformations					1		4		5	2.36	
Facial hemiatrophy and paralysis of VII nerve	1			1	1			1	4	1.89	
Subluxation of the hip	1		1		1		1		4	1.89	
Ichthyosis	2			1			1		4	1.89	
Deaf-mutism		1		1				1	3	1.42	
Cataract and deafness			1				2		3	1.42	
Gross brain defects and severe mental deficiency	1			1			1		3	1.42	
Pyloric stenosis		1	1	1					3	1.42	
Hypospadias	1		1				1		3	1.42	
Spina bifida and associated malformations	1				1			1	3	1.42	
Syndactyly (hands and feet)	1						2		3	1.42	
Congenital torticolis	1		1	1					3	1.42	
Epilepsy					2				2	0.94	
Hydrocephaly and multiple cranial malformations	1						1		2	0.94	
Oxycephaly and multiple defects		1					1		2	0.94	
Gothic palate	1		1						2	0.94	
Bilateral palpebral ptosis							2		2	0.94	
Endocrine disorders			2						2	0.94	
Klippel-Feil syndrome								1	1	0.47	
Turner's syndrome						1			1	0.47	
Paralysis	1								1	0.47	
Language disorder							1		1	0.47	
Anodontia and multiple defects	1								1	0.47	
Bilateral congenital absence of the ears (anotia)							1		1	0.47	
Malformations of the ear							1		1	0.47	

TABLE 9—*Continued*

Type of defect	I	II	III	IV	V	VI	VII	VIII	Total CHD Group	%	Control
Congenital absence of soft palate							1		1	0.47	
Craniopharyngioma								1	1	0.47	
Supernumerary salivary gland		1							1	0.47	
Infantile glaucoma			1						1	0.47	
Cervical rib	1								1	0.47	
Multiple exostoses							1		1	0.47	
Lipomatosis			1						1	0.47	
Severe hypotonia					1				1	0.47	
Hypertrophy of the clitoris					1				1	0.47	
Congenital stridor (laryngeal malformation?)											1
Total	36	9	22	20	26	4	70	25	212	100.00	5
Per cent	15.13	16.07	16.18	13.99	26.80	7.41	21.08	18.94	17.85		

G. Other congenital malformations.—

1. In the propositus.—Some 17.9 per cent of the propositi exhibited a second congenital malformation (Table 9). Among the common second defects observed were mongolism (generally in association with atrioventricularis communis), cataract and deaf mutism (often, presumably, on the basis of rubella), harelip and cleft palate, and angiomatosis. The frequency of multiple defects varies considerably from one clinical subgroup to the next. The greatest frequency noted was in Group V (atrial septal defect—26.8%), and lowest in Group VI (coarctation of the aorta —7.4%).

In studying the role of maternal age in the etiology of multiple defects, we noted that if cases of mongolism were not excluded, the frequency of multiple defect increased more or less linearly with mother's age up to ages 35–39, but in age group 40–44 the frequency has almost doubled over that observed in ages 35–39 (19.8%) (Table 11). If cases of mongolism were excluded, however, the frequency of multiple defect did not increase with maternal age, and was, on the average, 13.5 per cent.

In the control group, the frequency of primary defect is very low, 0.76 per cent, (5 cases of angiomatosis, 1 of harelip and cleft palate, and 1 of congenital stridor), and it was impossible to ascertain with any measure of reliability the frequency of multiple defect.

2. In the sibships of the propositi.—The frequency of malformations other than congenital heart disease was 0.98 per cent (20 out of 2,045 siblings). In the control group, this frequency was 1.09 per cent (9 out of 823) (Table 10). There is little evidence, then, for a difference between these two groups. The frequency of congenital malformations in the general population is not well known in France. An estimate of 1–2 per cent seems a good approximation yet a recent study by Wallace and co-workers (1956) gives, for New York, an overall incidence as low as 8.9 per 1000 live births. Therefore our finding of 1 per cent malformations other than CHD, in the siblings of the propositi, are not unusual.

3. In the parents of the propositi.—We have observed 10 cases in the CHD group

TABLE 10. DEFECTS (OTHER THAN CHD) IN THE RELATIVES OF THE PROPOSITI

Group	Sibship	Parents	1st cousins	Description of the defect
I	1	1		Hemophilia (father and brother)
		2		Harelip (father)
		1		Diabetes
		1		Hydrocephaly
			6	Mental retardation; harelip; other malformation
II				
III	2			Bilateral club foot
	1			Angiomatosis
	1			Congenital torticolis
		1		Diabetes
IV	1			Gross malformation of the abdomen
	1			Malformations of the hands
		1		Cleft palate
			1	Malformations of the hands
			2	Harelip
			4	Various malformations
V			1	Harelip and spina bifida
			1	Mental retardation
VI	1			Multiple defects
VII	1			Bilateral palpebral ptosis
	1			Hemogenia (sister)
	3			Encephalopathy
	1			Harelip
	1			Club feet
	1			Angiomatosis
	1			Mental retardation
		1		Harelip
			6	Harelip
			4	Various malformations
VIII	2			Club feet (brother and sister)
	1			Club feet
		1		Subluxation of the hip
		1		Diabetes
Total	20	10	25	
Controls	1			Mental retardation
	1			Harelip and cleft palate
	1			Hypospadias
	2			Barrel chest (two brothers)
	3			Mutism (three brothers)
	1			Idiocy
			1	Supernumerary hemivertebra

where one parent was malformed, with fathers and mothers equally often affected; in the control group, no cases of malformations among the parents were recorded (Table 10).

4. In the first cousins.—The frequency of congenital anomalies among the first cousins of the index cases cannot be given with accuracy because the total num-

TABLE 11. FREQUENCY OF OTHER DEFECTS IN THE PROPOSITI BY MATERNAL AGE WITH AND WITHOUT THE INCLUSION OF CASES OF MONGOLISM

Maternal age group	15–19	20–24	25–29	30–34	35–39	40–44	45+	Total
N	64	305	352	240	157	67	3	1188
Other defects	9	47	59	41	31	24	1	212
Per cent	14.06	15.41	16.76	17.08	19.75	35.82	33.33	17.84
Number of mongols	1	6	6	10	14	14	1	52
Per cent among non-mongols	12.50	13.44	15.05	12.91	10.82	14.92	0	13.46

TABLE 12 A DESCRIPTION OF THOSE CASES WHERE THE PROPOSITUS IS ONE OF TWINS OR TRIPLETS

Group	Index Case is a Male	Index Case is a Female
I		
II	DZ brother, died at 5 wks.; no cyanosis, but murmur and edema.	Triple birth (♀ ♀ ♂); the two twins died at 5 wks. from infection.
III		
IV	One MZ brother normal; one dead fetus expelled.	One sister of unknown zygosity died of accident. Normal.
	One brother of unknown zygosity. Normal	
	One MZ brother. Normal.	
V	One MZ brother. Normal.	Triple birth (♀ ♀ ♀); two fetuses expelled during pregnancy.
VI		One DZ sister; no CHD but convulsions.
		One brother. Normal.
VII	One sister. Normal.	One sister of unknown zygosity; died at 8 days.
	One sister. Normal.	
	One brother of uncertain zygosity, yet both are CcDE.	One MZ sister; no CHD but Little's disease.
		One sister died before birth.
		One sister MZ; normal.
		One sister of unknown zygosity.
VIII	One MZ brother. Normal.	One brother. Normal.
	One MZ brother. Normal.	
	One DZ brother. Normal.	
Control	One DZ brother. Normal.	One MZ sister. Normal.
	One brother of unknown zygosity, dead; premature, 6 months.	One MZ sister. Normal.
		One MZ sister. Normal.

ber of first cousins is not known. However, 25 cases of malformations have been observed in the CHD group and only 2 cases in the control group (Table 10). This might suggest a higher frequency in the CHD group.

H. Multiple births.—Among the 1,188 propositi there were 22 unrelated individuals resulting from multiple births of which 19 were twin births and 3 were triplet births (Tables 12 and 13). In the control population, we observed 5 cases of twinning among the 660 propositi and no cases of triplets. The frequency of twins in both groups is low when compared to the frequency of twins at birth. This fact may be explained by our method of ascertainment which includes only children

TABLE 13. CONCORDANCE OR DISCORDANCE WITH RESPECT TO CHD IN MULTIPLE BIRTHS BY CLINICAL SUBGROUP

Group	ZYGOSITY								
	MZ			DZ			? Dx		
	Concordant	Discordant	?	Concordant	Discordant	?	Concordant	Discordant	?
I									
II				1	1*				
III									
IV		2*			1			1	
V		1							1*
VI					2				
VII		2			2			1	3
VIII		2			2				
Total: 22.....	0	7	0	1	8	0	0	2	4

* = Triplets.

having passed the early years of life in which mortality is known to be higher among individuals from multiple births than among those from single births. This bias, due to ascertainment, is introduced in both the CHD and control groups, hence we may justifiably compare them with regard to the frequency of twins and triplets. The total X^2 computed with Yates' correction is equal to 3.30, with two degrees of freedom and $P = 0.20$. Thus the frequency of multiple births is not elevated in the CHD group when compared to the control. Yet the frequency of triplets is high, and this may suggest a predisposition to malformation in multiple births involving more than two individuals.

Further study of the twins reveals the following: (a) Of seven presumably MZ twin pairs diagnosed by the usual morphological criteria in no instance were both twins affected, and (b) of the nine DZ twin pairs in only one instance was the co-twin affected. In six cases, a precise diagnosis of the type of twinning was not available. Among the latter pairs, two showed discordance, and four were uncertain because of neonatal death of the co-twin of the propositus.

DISCUSSION

A. The role of genetic factors in the etiology of CHD.—Sex ratio, for convenience, may be discussed here although association with sex does not necessarily imply genetic determination. Females more frequently than males have patent ductus arteriosus (3 girls to 1 boy, on the average) or moderate truncular pulmonary atresia (9 girls to 1 boy). It is interesting to note that these defects may be embryologically related, since the first involves the distal end of the sixth aortic arch and the other the proximal portion of the same arch. Aortic malformations appear to be more frequent in males, particularly coarctation of the aorta. In this connection, Polani and Campbell (1955) have observed a significantly higher frequency of males with Fallot's tetralogy. Our data suggest a similar trend but the increase is not statistically significant. The reason for these particular discrepancies with regard to the sex ratio is not clear since the sex ratio in the total sample is normal. As Polani and Campbell have pointed out, the hypothesis of a differential fetal death rate in the two sexes raises more questions that it resolves.

The general frequency of congenital heart disease is not known in France, but if we accept Polani and Campbell's English estimate of 0.1 per cent, the frequencies observed in the siblings of the CHD propositi are ten, twenty, and even thirty times higher depending upon the clinical subgroup. Specifically, we find that the frequency of heart malformations among the siblings of the propositi is, on the average, 1.47 per cent, and is noticeably elevated in pulmonary valvular stenosis (3.74%) and in coarctation of the aorta (2.56%). Even if the general frequency of CHD is higher, 0.7 per cent as Anderson has suggested, we note an increase which is twofold in the entire CHD group and may be as high as sevenfold in clinical subgroups. No striking elevation is observed in I.V. septal defect or Fallot's tetralogy. It must be emphasized, however, that in 23 cases out of 30 the clinical type of heart malformation exhibited by the propositus is not the same as that of his affected sibling. This suggests that if the high familial incidence of congenital heart disease is real, there is little in the way of intra-family specificity of type of malformation.

Consanguineous marriages are significantly more frequent in the CHD group than in the control, the latter reflecting the incidence in the general population. The consanguinity rate varies in the different clinical subgroups, being highest in pulmonary valvular stenosis and atrial septal defect, and lowest in Fallot's tetralogy, interventricular foramen, and coarctation of the aorta.

The siblings of the propositi from the consanguineous matings exhibit an elevated incidence of heart malformations, but this appears merely to reflect an increase in the over-all rate of malformations. This finding is in agreement with our observations that (1) in the event of multiple cases of heart malformations in a family, the malformations are dissimilar in type, and (2) the frequency of multiple defects in the propositus as well as his siblings is increased.

The 22 cases of twins here reported hardly support the hypothesis that congenital heart diseases are hereditary. Of seven MZ pairs, none showed concordance, while out of nine DZ pairs, one showed concordance.

In summary, certain findings, namely, the high consanguinity rate and the elevated familial incidence, suggest the importance of hereditary factors in the etiology of CHD, but the familial incidence is of a nonspecific variety. Nevertheless, as will be discussed later, this is evidence for ultimate genetic determination.

B. The role of non-genetic factors in the etiology of CHD.—Non-genetic factors such as maternal age, birth rank, irregularities during pregnancy, and time of year of birth, have been deemed important in the etiology of CHD by a number of authors (reviewed in the introduction). Our data provide further information with regard to these factors.

In agreement with Polani and Campbell but in disagreement with Richards, our analysis of maternal age failed to reveal a significant effect on the incidence of congenital heart disease. The birth rank data, however, reveal a significant difference, the mean birth rank being slightly higher in the CHD than in the control group.

The frequency of any irregularity during pregnancy is twice as high in the CHD group as in the control group. When we inquire into the sorts of irregularities which occur, we find that viral infections in general, but rubella in particular, are common events during the course of pregnancy in the CHD group. Moreover, the threat of interruption of pregnancy generally in the first three months is appreciably more common (two times) in the CHD than in the control group. This raises a number of questions with regard to cause and effect relationships assuming, of course, that the observed difference is real and that cause and effect relationships do exist. For example, we might ask whether or not the threat of interruption represents a "ma-

ternal defense" mechanism. In this connection, we note that among older mothers (over 35) the frequency of these accidents is only half that to be found in younger mothers. We might wonder whether this means that older mothers react less or whether factors provoking a threat of pregnancy interruption in young mothers lead to complete miscarriages in older mothers. Either of these alternatives could account for our observations. Irrespective of their interpretation, these observations question the advisability of interfering with the course of a spontaneous miscarriage.

Rutstein et al. claimed, on a sample of approximately 12 cases of patent ductus arteriosus, that a higher incidence of patent ductus occurred during the winter months. The distribution of CHD cases by month of birth showed no seasonal effect in our sample but lack of reliable figures with regard to the seasonal distribution of rubella in France did not allow us to draw any conclusions with regard to this point. The frequency of twins was not elevated in our data, yet we observed 3 cases of triplets. Some authors have suggested a cause and effect relationship between multiple births and malformations. Morison believes that there exists a vital competition between twins at a very early embryonic stage (chorio frondosum). One consequence of this competition may be lack of nutrition in one of the twins, which, if severe, may lead to a papyraceous fetus or if less severe, to an anomaly in development. The fact that we have not observed concordance in MZ twins suggests that if genetic factors are important, the vital competition is the "triggering" mechanism. Otherwise stated, if genetic factors and vital competition are essential to the formation of an anomaly of the heart, then one would expect MZ twins to be more often discordant than concordant.

Evidence from domestic animal experimentation suggests that situations similar to those described by Sawin and co-workers (1947, 1949) in rabbits may exist in man. The frequency with which kyphoscoliosis and chest deformities are observed among the propositi (10% of cases) would support this view. Lerner's (1954) and Landauer's (1952) findings in sporadic malformations in poultry have also their counterpart in our findings of sporadic distribution, high consanguinity rate, and high familial incidence in CHD.

To explain all our findings, one must assume that the genetic factors are complex and not specific. These factors would presumably influence embryonic growth by acting upon the "growth areas" described by Sawin and Edmonds in rabbit embryos. The nature of the resulting malformation would be a function of environmental conditions and the neighboring "growth areas."

D. Conclusions.—The importance of the various possible etiological factors for each of the clinical groups may be crudely weighed. In Fallot's tetralogy, genetic factors appear less important than non-genetic factors since consanguinity and familial incidence are both low, and irregularities of pregnancy, etc., are common occurrences. In pulmonary stenosis, however, genetic factors appear more important because of the high consanguinity rate and high familial incidence, and the low frequency of irregularities during pregnancy, etc. In patent ductus arteriosus, genetic factors appear somewhat less important since there is neither a marked increase in consanguinity, nor familial incidence, and abnormalities during pregnancy are relatively common. In I.V. septal defect, non-genetic factors appear to be the paramount cause of the defect. In atrial septal defect, genetic and non-genetic factors appear equally important. No judgment can be made with regard to coarctation of the aorta because of the paucity of data.

The frequency of associated malformations in the propositi does not alter these

TABLE 14. RELATIVE IMPORTANCE (INDICATED BY NUMBER OF + SIGNS) OF GENETIC AND NON-GENETIC FACTORS IN THE ETIOLOGY OF THE FIVE MOST COMMON CLINICAL SUBGROUPS

(Groups VII and VIII are not included because of their lack of homogeneity; Group VI is not included because of the small number of cases.) See text for further explanation.

Clinical subgroups	Genetic Factors		Non-genetic Factors	Frequency of associated malformations
	Consanguinity	Incidence of CHD in the sibships	Abnormalities during pregnancy	
Fallot's tetralogy	+	+	++++	++
Pulmonary valvular stenosis	++++	++++	+	++
Patent ductus arteriosus	++	++	++++	++
I.V. septal defect	+	+	++++	++
Atrial septal defect	+++	++	+++	++++

judgments since in the first four groups, where genetic and non-genetic factors vary appreciably in relative importance, the frequency of associated defect is unchanged. This might be interpreted as meaning that the associated defect is frequently merely a secondary developmental phenomenon. These observations are summarized in Table 14.

SUMMARY

The families of 1,188 propositi with congenital heart disease (CHD) diagnosed in the Hopital des Enfants Malades, Paris, France, were compared with the families of a control group of 660 children randomly selected.

1. In the entire CHD group, the sex-ratio was not significantly different from a theoretical 1:1 sex-ratio nor from that observed in the control group. But the sex-ratio was not uniform in all clinical subgroups. In patent ductus arteriosus the frequency of females was significantly higher than that of males (on the average 3:1). The frequency of males is higher than females in valvular aortic stenosis (7:1, 16 cases). In moderate truncular pulmonary atresia, the frequency of affected females is higher (9:1, 10 cases).

2. The mean maternal age for the CHD group, in its entirety, did not differ significantly from that for the control group (28.39 vs. 28.84 years). Mean birth rank is significantly higher ($t = 2.92$) in the CHD group than in the control group (2.23 vs. 2.03).

3. The average coefficient of relationship of the parents of the propositi was 3.6 times higher in the CHD group than in the control group. Considerable variation in the average coefficient of relationship was observed in the different clinical subgroups. Further evidence suggested that consanguinity increases the frequency of all malformations and, as a consequence, the frequency of CHD.

4. The frequency of infection, threats of miscarriage and premature delivery, and other abnormalities during pregnancy, was twice as high in the CHD group as in the control. Threats of interruption of pregnancy are twice as frequent among mothers under 35 years of age giving birth to children with CHD as among mothers over 35 years with similarly affected infants.

5. The frequency of CHD among the siblings of the propositi (1.46 per cent) was higher than that observed in the control group or in the general population. It was, however, not that expected for a single-gene hypothesis. In familial cases of CHD, the clinical type of heart disease is frequently dissimilar in the propositus and the affected relative.

6. Eighteen per cent of the propositi exhibited a second congenital malformation.

7. The frequency of multiple births among the propositi born with CHD was not different from that observed in the control group. All seven pairs of MZ twins showed discordance with regard to CHD, while one out of nine pairs of DZ twins showed concordance.

8. These results suggest that both genetic and non-genetic factors play a part in the etiology of CHD. The relative importance of genetic and non-genetic factors appears to vary substantially from one clinical subgroup to another.

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Cytologic Maps of Some Isolated Human Pachytene Chromosomes[1]

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INTRODUCTION

SCHULTZ AND ST. LAWRENCE (1949) were the first to report the applicability of the aceto-carmine squash technique in approaching a most challenging task—the cytologic mapping of a human pachytene bivalent. Their observations on the "nucleolar chromosome" encouraged this writer to try similar mappings of some of the remaining autosomal pairs. Recently, Kodani (Ref. Neel and Schull, 1954)[2] completed the chromomere pattern of the "second nucleolar chromosome". Both studies utilized persisting nucleoli which are associated with these chromosomes as morphologic markers. When present, nucleoli are helpful in locating bivalents or component parts among the overlapping members of a squashed complement.

Some aspects of the procedures to be described may be regarded as destructive to cytologic structures. The appearance of the "nucleolar chromosome", as described by Schultz and St. Lawrence, served as the basis for determining the accuracy of the method. It was found to be satisfactory. By exercising control over the instruments used to isolate autosomal bivalents, nine distinct types have been analyzed in some detail.

MATERIALS AND METHODS

Isolation Procedures

Tissue was obtained from an eighteen year old white male a few hours after he had succumbed to tetanus. The gonads were cubed before they were placed in alcohol-acetic (3:1) fixative and refrigerated in fresh fixative for approximately twenty-four hours. Connective tissue was removed prior to placing clusters of seminiferous tubules in a Waring blendor. A sufficient volume of unmordanted 0.5–1.0 per cent aceto-carmine was added to bring the tip of the single downward-pointing blade of the rotary knife in contact with the surface of the stain. By limiting the volume to this level, the destructive homogenizing action of the single blade was minimized. As an added precaution, centrifugal agitation resulting from the downward-pointing blade was reduced by turning the motor on and off repeatedly during the procedure.

Agitation by the blendor and the softening action of acetic acid caused the

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[2] Figure 2–4 of Neel and Schull (1954) is in reverse order. Chromosome 1 of Schultz and St. Lawrence possesses the larger nucleolus (lower figure).

seminiferous tubules to disassociate within a few minutes. The course of this process was followed every fifteen seconds, by pipetting drops of the "suspension" and observing them under the microscope as temporary mounts. This periodic check provided a simple and rapid means of following the unravelling of many intact and unstretched pachytene bivalents. An exact chromosome number cannot be ascertained by these means because the nuclei are entirely disrupted.

Separation of the suspended components into several fractions was facilitated by a clinical centrifuge operated at half speed for approximately two minutes. In this period, fatty globules rose to the surface; bits of tubules and clumps of intact sertoli and connective cells made up the debris; the desired middle layer contained numerous isolated bivalents. The fat globules were decanted and the middle layer transferred for recentrifugation; the remaining debris was pooled in a few milliliters of dilute (0.5 per cent) unmordanted stain and refrigerated in a paraffin-sealed vial.

Recentrifugation of the middle layer (the stain containing isolated chromatin) was repeated at least four times, each followed by decanting the stain and pooling the sediment from several test tubes. Successive centrifugations were lengthened by approximately two minutes to insure the collection of smaller bivalents. The second and third samples contained the least amount of cytoplasmic debris and remained in excellent condition for many months when refrigerated in dilute unmordanted stain.

Preparation of Slides

Material for a temporary slide was drawn from the sediment with a fine pipette. Deeper staining was attained with a small drop of 2.0 per cent aceto-orcein, or by mordanting, with a clean dissecting needle for 15 or 20 seconds, an added drop of 1 per cent aceto-carmine and the sample. After setting the cover glass, the slide was pressed very gently under many layers of bibulous paper and then sealed. At times, smaller bivalents were disturbed by Brownian movement, thus requiring added pressure. This was exerted by a small lead weight left in place for several hours.

Photographs were taken while slides were temporarily sealed. The initial magnification of 1530 × on the negative was obtained with a 60 × apochromatic objective, 15 × Mobini ocular, and bellows extended to provide an enlarging factor of 1.7 ×. Kodak contrast process panchromatic film, and a No. 87 Wratten filter were used at all times.

Camera Lucida

Approximately 250 camera lucida drawings were made of randomly selected bivalents in order to become acquainted with the more prominent morphological features of intact pairs. These illustrations also provided a preliminary reconstruction of the autosomal pairs. Drawings were placed in one of twenty-eight arbitrary groups by comparing and matching intact appearing telomeres and interstitial segments. Five of these groups consisted of fragments induced during some phase of the isolation procedure, two represented portions of bivalents that repeatedly separated near large, dense chromomeres, and the remaining groups consisted of unidentifiable fragments which, to some extent, had similar chromomere patterns.

DESCRIPTION OF THE BIVALENTS

Schultz and St. Lawrence's Nucleolar Chromosome

Aside from the lack of an exact or replicable chromomere count due to the unstretched state, present examples of the nucleolar bivalent resemble those described earlier by Schultz and St. Lawrence. Intact bivalents with medially placed nucleoli and dense chromomeres on either side (Figures 1 A–C) and separate "arms" with attached nucleoli (Figure 1 D–G) were noted with equal frequency. The persistent absence of longitudinal stretching (judged by the natural appearance of the "arms" and the resulting proximity of chromomeres) and adherence of nucleoli (medially or terminally) suggest that the mechanical procedures employed were no more disturbing to this particular bivalent than that which may be expected from squashing intact cells or seminiferous tubules.

The Remaining Bivalents

The more striking morphologic features noted among the nine bivalents selected for presentation at this time are: (a) limited longitudinal stretching as compared to squash preparations; (b) large, positively heterochromatic, or densely stained knobs dispersed among numerous smaller and more lightly stained chromomeres; (c) moderately long segments of chromatin composed of lightly stained chromomeres; (d) minute "trabant-like" telomeres or chromosome ends; (e) individual forms or shapes assumed by many bivalents after being released from the bounds of the nuclear membrane; and (f) minimal lampbrushing of pachytene elements. Stretching was so slight that semi-diagrammatic drawings provided a further check for detecting mechanical disturbances among bivalents that lack characteristic telomeres and interstitial segments. Reference to any of the accompanying text-figures will illustrate the slight variations in length that occurred. Although these features prevented an accurate chromomere count for each of the bivalents, many fine details, such as the trabant-like telomeres, were left undisturbed and, in turn, served as markers for identification in the absence of nucleoli.

Bivalent 1

The longest pachytene structure in this series had 19–24 uniform and evenly spaced chromomeres (Figure 2). At first, differences in chromomere counts were thought to be the result of slight longitudinal elongation causing closely associated chromomeres to separate into more distinct units. However, upon completion of semi-diagrammatic drawings, it became evident that this was not the case, since shorter bivalents had at least as many chromomeres as the longer representatives. In this study variations in chromomere number were more likely the result of one or more technical procedures other than elongation. For example, the degree of staining and swelling of chromomeres was readily enhanced by excessive mordant or aging of the slide, thus leading to "thickening" or coalescence of neighboring chromatins. This is exemplified by the two bivalents in Figures 2 E and F, in which the chromomeres appear to be fewer in number in segments that are deeply stained and swollen. This bivalent, the longest in the complement, has a tendency to straighten when released from the bounds of the nuclear membrane, as illustrated in Figures 2 C and D.

FIGURE 1. "NUCLEOLAR CHROMOSOME" DESCRIBED BY SCHULTZ AND ST. LAWRENCE: A, B AND C. INTACT BIVALENTS WITH MEDIAL NUCLEOLUS BETWEEN DENSE CHROMOMERES. D–F. LONG "ARM" OF THE NUCLEOLAR CHROMOSOME BEARING NUCLEOLUS IN A TERMINAL FASHION DUE TO SEPARATION AND LOSS OF SHORTER "ARM". 3060 ×.

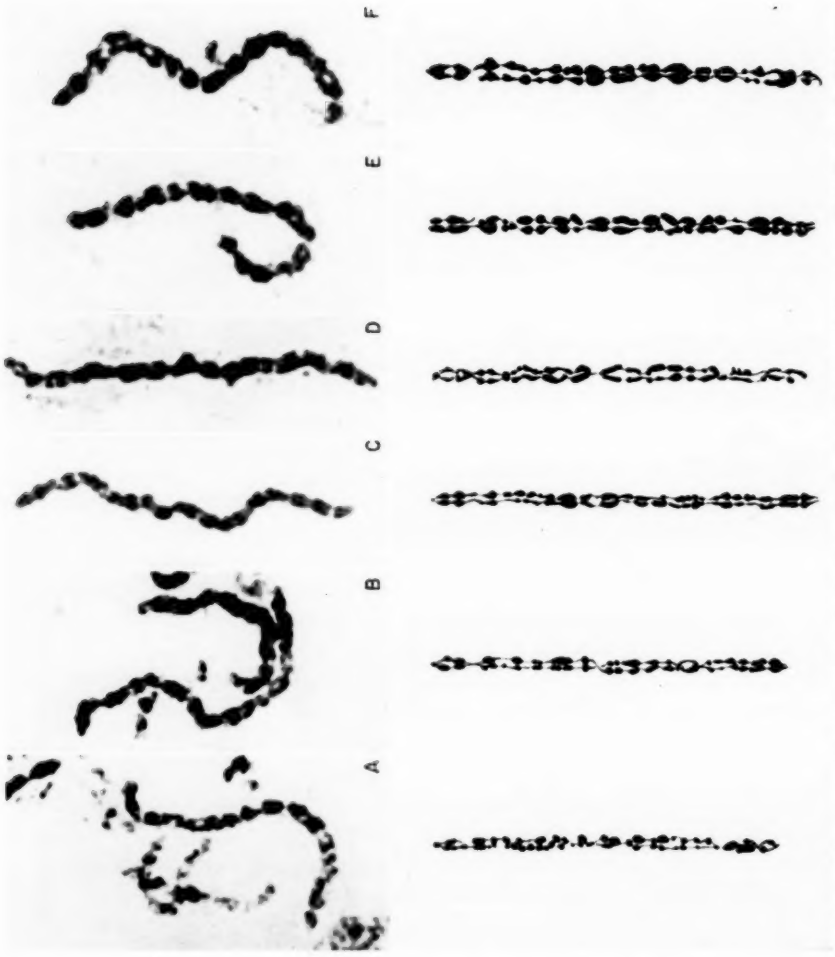

FIGURE 2. CHROMOSOME 1 OF SERIES: NOTE ABSENCE OF DISTINCT SEGMENTS OR TELOMERES AND STRAIGHT APPEARANCE. 1500 ×.

Bivalent II

Three large and prominent chromatic knobs are set apart by segments which, contrastingly, contain the smaller chromomeres (Figure 3). Some, in the lighter portions, are slightly elongated, while others are dot-like in appearance. These elements form a striking and easily identified pattern. There are approximately 26 chromomeres, in addition to the three large knobs in this chromosome. The quadripartite or 4-stranded nature of the pachytene bivalent is evident in the representative that displays medial asynapsis (Figure 3D).

This bivalent was readily fragmented during the isolation procedures. Generally, breaks occurred in the lightly staining segments on either side of the dense knobs. The resulting fragments (Figures 5 E and F) resemble Bivalent IV (Figures 5 A–D). They form one of the five extra sets of camera lucida drawings mentioned above.

Chiasmata or other evidence of crossing-over were never encountered among bivalents in mid-late pachytene. Distal portions of the asynaptic region in Figure 3 D also lack structural details that would indicate crossing-over or an early stage of terminalization of chiasmata. Comparison with the figures in Hsu (1952), and Tjio and Levan (1956) suggest Bivalent II to be one of the five largest in the complement.

Bivalent III

The characteristic form of this bivalent is that of a "boomerang". Most representatives have prominent constrictions due to twisting of the paired homologues (Figure 4 C, D, E, and F). There are 15–20 distinct chromomeres in this chromosome. They are comparable in size to those of Bivalent I.

Bivalent IV

This bivalent is readily identified by a dense, medially positioned chromatic knob, flanked on either side by approximately equal lengths of diffusely staining chromatin (Figures 5 A, B, C, and D). There are 6–8 smaller chromomeres in each of the diffuse segments or "arms". Figures 5 E and F are fragmented portions of Bivalent II.

Bivalent V

Generally, this bivalent assumed a J-shaped form when released from the nuclear membrane (Figures 6 A, B, and C). Dense telomeres attached to filamentous strands are prominently set apart from proximal chromatin. Twisting of the bivalent is evident in regions having clear, elongated segments of chromatin (Figures 6 B and C). Some fifteen distinct chromomeres are seen in the unstretched bivalent.

Bivalent VI

This C-shaped or "duck-billed" bivalent features 17–20 chromomeres and trabant-like projections from one of the telomeres (Figures 6 E and F). The latter structures illustrate the delicacy of some human chromosomes and serve as secondary morphologic markers.

Bivalent VII

The varied appearance of the uppermost telomere and adjacent chromomere (Figures 7 A and B) is probably due to a 90° rotation in the immediate interchromeric

FIGURE 2. CHROMOSOME 1 OF SERIES; NOTE ABSENCE OF DISTINCT SEGMENTS OR TELOMERES AND STRAIGHT APPEARANCE. 1500 ×.

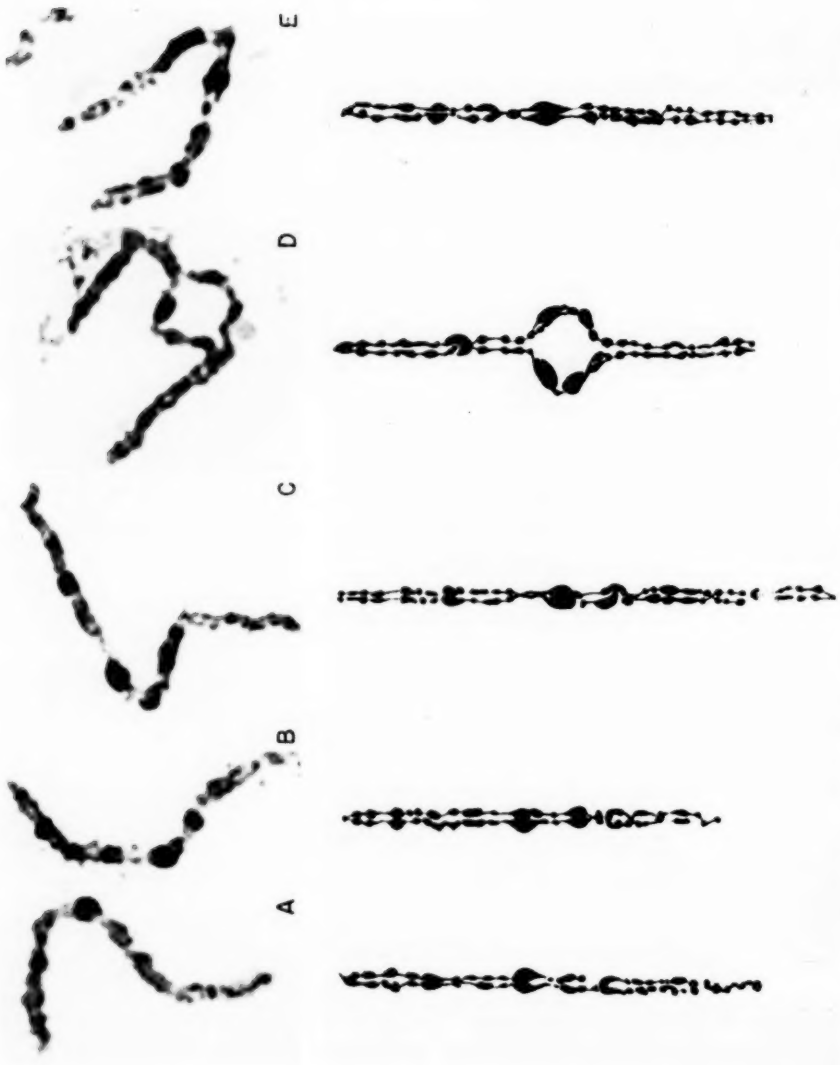

FIGURE 3. CHROMOSOME II OF SERIES: DENSE, CHROMATIC KNOBS EVIDENT IN ALL REPRESENTATIVES. D. QUADRIPARTITE NATURE OF BIVALENT SEEN IN ASYNAPTIC REGION. 1940 ×.

FIGURE 3. CHROMOSOME II OF SERIES: DENSE, CHROMATIC KNOBS EVIDENT IN ALL REPRESENTATIVES. D. QUADRIPARTITE NATURE OF BIVALENT SEEN IN ASYNAPTIC REGION. 1940 ×.

FIGURE 4. CHROMOSOME III OF SERIES: NOTE BOOMERANG-LIKE APPEARANCE. 3060 ×.

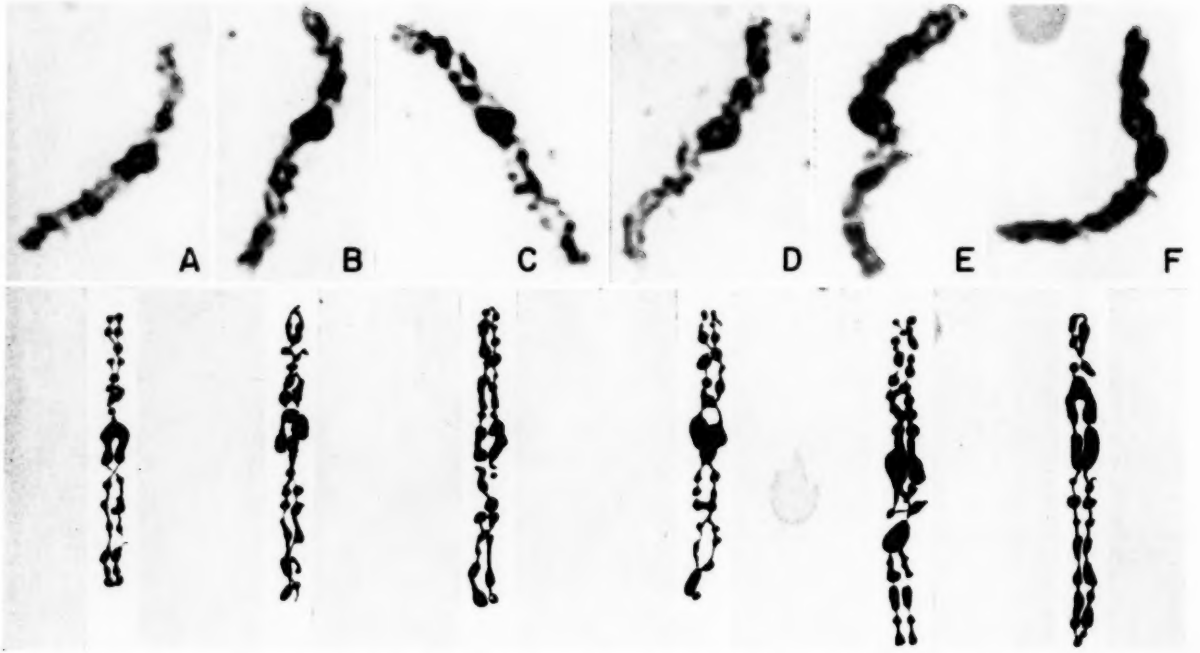

FIGURE 5. A, B, C, AND D. CHROMOSOME IV OF THIS SERIES: NOTE DENSE MEDIAL CHROMOMERE OF KNOB. E AND F. FRAGMENTED MEDIAL SEGMENTS OF CHROMOSOME II THAT SUPERFICIALLY RESEMBLE CHROMOSOME IV. 3060 ×.

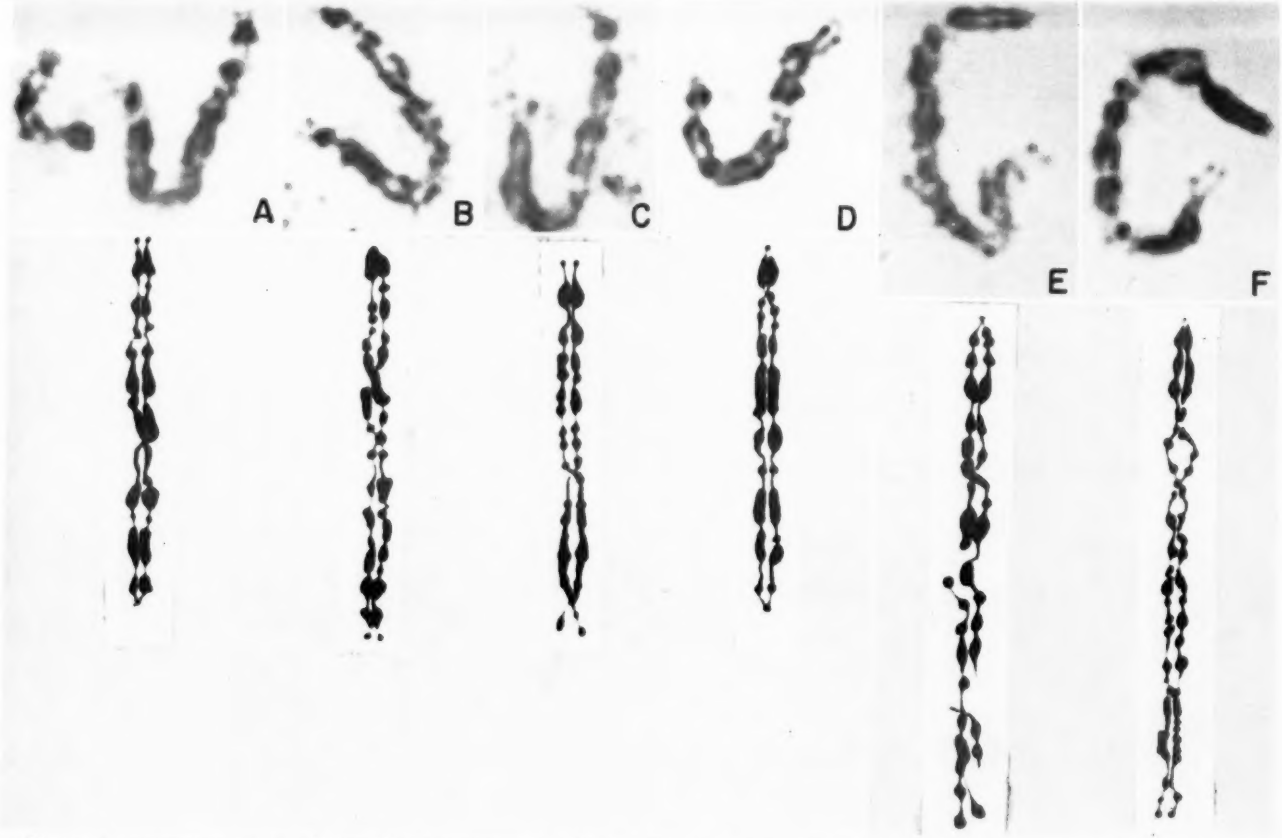

FIGURE 6. CHROMOSOME V (A, B, C, AND D) AND CHROMOSOME VI (E AND F) OF THIS SERIES. NOTE THE J-SHAPED REPRESENTATIVES OF CHROMOSOME V, AND THE CHARACTERISTIC "HEAD" AND TRABANT-LIKE TELOMERES OF CHROMOSOME VI. 3060 ×.

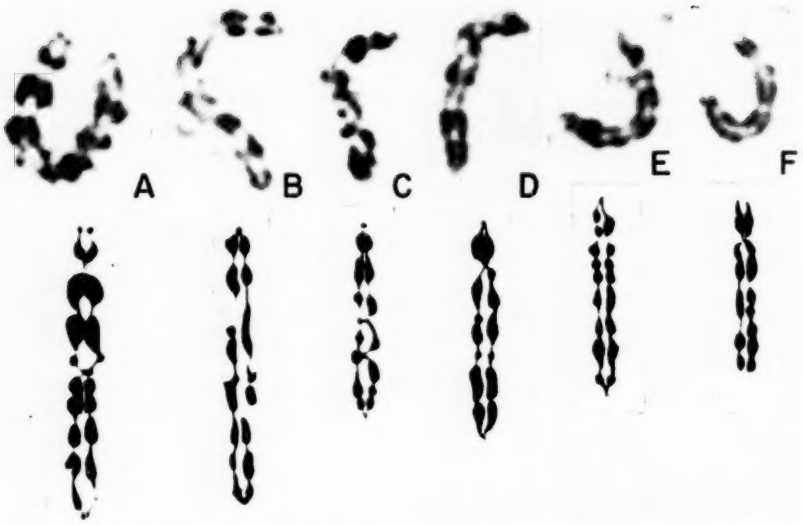

FIGURE 7. CHROMOSOMES VII (A AND B), VIII (C AND D), AND IX (E AND F) OF THIS SERIES. 3060 ×.

spaces. The dot-like upper pair of telomeres in (A) are seen as a single unit in (B): the former appearance represents paired homologues; the latter is thought to be the more closely associated sister strands. The apparent differences in the widths of the chromomeres in the two samples are attributed to differences in pressure exerted while preparing the slide. This chromosome has approximately eleven chromomeres.

Bivalent VIII

The major features are a constriction and a bend proximal to the large chromomere at the upper end and a dot-like medial chromomere (Figures 7 C and D). This bivalent has about nine chromomeres, ranging from small dot-like forms to dense elongated chromomeres.

Bivalent IX

This is the smallest bivalent of the series. It has a distinct subtelomeric constriction separating one of the eight unstretched chromomeres from the other seven (Figures 7 E and F). The U-shaped form is also characteristic of this structure.

DISCUSSION

Recent descriptions of mammalian late pachytene chromosomes have been concerned primarily with structural appearances and the mapping of chromomeres. The chromosomes of the house mouse, *Mus musculus* have been described by Slizynski (1949, 1952) and Griffen (1955) as having vesiculate and punctate chromomeres, giving the impression that the elongated 4-stranded bivalent is composed of "lines" or bands like those of the polytenic or multi-stranded Dipteran salivary gland chromo-

somes. On the other hand, the chromosomes of the Chinese hamster, *Cricetulus griseus* during early and mid-pachytene, exhibit a corpuscular chromomere pattern, made up of extremely spiralled chromonemata and ranging in size from dot-like spheres to large dense chromatic knobs similar in appearance, except for a moderate amount of lampbrushing, to those observed in man and numerous species of grasses and liliaceous plants. Although centromeric regions of the ten autosomal pairs of the Chinese hamster are clearly evident during late pachytene, similar sites in man and the mouse are difficult to detect with any degree of accuracy. Several reports on the mouse place the position of centromeres subterminally (Levan and Hauschka, 1953; Hungerford, 1955; and Griffen, 1955) in contrast to the medial locations of about one dozen centromeres reported earlier by Slizynski (1949).

The appearance of chromosomes, notably in metaphase, is determined by the position of the centromere, lengths of the arms and the degree of spiralization. Any attempt to relate submicroscopic alterations of fixed nucleoproteins to the forms assumed by isolated chromonemata is, of course, purely speculative. Reference to this phenomenon is limited to reiterating that in lieu of other striking morphologic features, these forms were exceedingly helpful in making gross identifications.

Preservation of delicate trabant-like extensions of telomeres as noted for Bivalents V and VI indicates the completeness retained by some structures. On the other hand, Bivalent II was readily fractured, as was the first nucleolar chromosome. Approximately half of the human complement of chromosomes, including the X and Y sex elements, have been accounted for in the pachytene stage of meiosis.

Aside from the limitation of obtaining fresh tissue whenever desired, the mapping of human pachytene bivalents requires less effort and time than that needed to conduct comparable trials with rodents having almost the same number of chromosomes. This is indeed noteworthy, considering the amount of time devoted in general to plant, amphibian, and rodent chromosomes, as compared to human chromosomes prepared from equally favorable tissues.

SUMMARY

Morphologic components of nine isolated human pachytene bivalents are described. Each bivalent is characterized by a distinct and recognizable form assumed after being released from the nuclear membrane. In the absence of chromosomal lampbrushing, large chromatic knobs, diffuse segments of chromatin, and in some cases, attached nucleoli, make up the general features for identifying these particular bivalents. Schultz and St. Lawrence's (1949) description of the "nucleolar chromosome" was confirmed.

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Further Scoring Types in Sequential Linkage Tests, With a Critical Review of Autosomal and Partial Sex Linkage in Man

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INTRODUCTION

THIS IS the fourth and last in a series of papers on the application of probability ratio (lod) scores to human linkage. The first (Morton, 1955a) considered the properties of sequential linkage tests and the scoring types which arise when the parental genotypes are known and there are only two alleles at each locus. The second (Morton, 1956) treated pedigree data and used lods to obtain likelihood ratio tests of homogeneity and maximum likelihood estimates of linkage. The third (Steinberg and Morton, 1956) included applications to multiple allelic test loci. The present communication extends the scores to multiple alleles at both loci, pseudoalleles, and partial sex linkage, and concludes with a review of possible linkages in man.

INTERCROSSES AND MULTIPLE ALLELES. PARENTAL GENOTYPES KNOWN, BOTH PARENTS TESTED. COMPLETE PENETRANCE, NO NATURAL SELECTION

In the absence of epistasis, all single and double backcrosses can be reduced to diallelic scoring types. For example, if T_1 and T_2 are codominant and t is recessive to T_1, so that the mating $T_1t \times T_1T_2$ produces progeny of phenotypes T_1, T_1T_2, and T_2t, then the mating Gg $T_1t \times$ gg T_1T_2 is of type 1 (Morton, 1955a) with the T_1 progeny not informative, and is scored as $z_1 + c_1$ or $z_1 + e_1$ with a(GT_1T_2), b(GT_2t), c(gT_1T_2), d(gT_2t), while the mating Gg $T_1T_2 \times$ Gg tt is of type 11 and is scored as z_2 with a(GT_1), b(gT_1), c(GT_2t), and d(gT_2t).

Multiple allelism introduces several new double intercrosses. The most important intercross types may be denoted by their segregation ratios as follows (Smith, 1954):

Type	Mating	Examples: ABO	Examples: MNS
31	$Tt \times Tt$	AO × AO	MS/Ms × MS/Ms
121	$T_1T_2 \times T_1T_2$	AB × AB	Ms/Ns × Ms/Ns
211	$T_1t \times T_1T_2$	AO × AB	MS/Ms × MS/Ns
1111	$T_1T_2 \times T_3T_4$ ($T_1T_2 \times T_2t$, etc.)	$A_2B \times A_1O$	MS/Ns × Ms/NS

These four intercrosses give eleven essentially different double intercrosses (table 1), of which the first five have been treated previously (Morton, 1955a; Steinberg and

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TABLE 1. DOUBLE INTERCROSSES

Mating class	Parental genotypes	Progeny					
(31)(31)	Gg Tt × Gg Tt		T	t	Sum		
		G	a	b	s_1		
		g	c	d	s_2		
(31)(121)	Gg T_1T_2 × Gg T_1T_2		T_1	T_2	T_1T_2		
		G	a	c	e		
		g	b	d	f		
(121)(121)	G_1G_2 T_1T_2 × G_1G_2 T_1T_2		T_1	T_2	T_1T_2		
		G_1	a	b	g	u = a+d	
		G_2	c	d	h	v = b+c	
		G_1G_2	e	f	i	w = e+f+g+h	
(31)(211)	Gg T_1t × Gg T_1T_2		T_1	T_2t	T_1T_2	Sum	
		G	a	c	e	s_1	
		g	b	d	f	s_2	
(31)(1111)	Gg T_1T_2 × Gg T_3T_4		T_1T_3	T_2T_3	T_1T_4	T_2T_4	Sum
		G	a	c	e	g	s_1
		g	b	d	f	h	s_2
(121)(211)	G_1G_2 T_1t × G_1G_2 T_1T_2		T_1	T_2t	T_1T_2	Sum	
		G_1	a	d	g	s_1	
		G_1G_2	—	e	h	s_2	
		G_2	c	f	i	s_3	
(121) (1111)	G_1G_2 T_1T_2 × G_1G_2 T_3T_4		T_1T_3	T_2T_3	T_1T_4	T_2T_4	Sum
		G_1	a	d	g	j	s_1
		G_1G_2	b	e	h	k	s_2
		G_2	c	f	i	l	s_3
(211)(211) cis	G_1g T_1t × G_1G_2 T_1T_2		T_1	T_2t	T_1T_2		
		G_1	a	d	g		
		G_2g	b	e	h		
		G_1G_2	c	f	i		
(211)(211) trans	G_1g T_1T_2 × G_1G_2 T_1t		T_1	T_2t	T_1T_2		
		G_1	—	d	g		
		G_2g	b	e	h		
		G_1G_2	c	f	i		
(211) (1111)	G_1g T_1T_2 × G_1G_2 T_3T_4		T_1T_3	T_2T_3	T_1T_4	T_2T_4	
		G_1	a	d	g	j	
		G_2g	b	e	h	k	
		G_1G_2	c	f	i	l	
(1111) (1111)	G_1G_2 T_1T_2 × G_3G_4 T_3T_4		T_1T_3	T_2T_3	T_1T_4	T_2T_4	
		G_1G_3	a	e	i	m	
		G_2G_3	b	f	j	n	
		G_1G_4	c	g	k	o	
		G_2G_4	d	h	l	p	

Morton, 1956). Several of these types are likely to be selected through the progeny for both loci, and this necessitates a score correction. The main locus G may be subject to truncate or arbitrary selection and the test locus T to truncate selection. In the 211 mating $T_1t \times T_1T_2$, selection will usually require at least one T_2t child ($T_2t > 0$). For example, in the absence of s-antisera or pedigree information, the mating MS/Ms × MS/Ns can be recognized only if there is at least one MNs child, and $A_1A_2 \times A_1B$ can be recognized only if there is at least one A_2B child. In the 1111

intercross type ($T_1T_2 \times T_3T_4$), the condition for selection may be $T_1T_4 + T_2T_4 > 0$ (for example, at least one A_2 or A_2B child from the mating $A_2B \times A_1A_2$, or at least one Ms or MNs child from Ms/Ns × Ms/NS). Another common condition for selection is $T_1T_4 + T_2T_4 > 0$, $T_2T_3 + T_2T_4 > 0$ (as at least one A_2 or A_2B child and

TABLE 2. DOUBLE INTERCROSS SCORES FOR THE PARENTS SEPARATELY

Mating class	Genotype	Selection		Score
		G	T	
(31)(211)	Gg T_1t	$g > 0$	$T_2t > 0$	$z_2(c, d, e, f) + c_2(c + d + e + f)$
(31)(211)	Gg T_1t	arbitrary	$T_2t > 0$	$z_2(c, d, e, f) + e_2(c + e, d + f)$
(31)(211)	Gg T_1T_2	arbitrary	complete	$z_2(a, b, c + e, d + f)$
(31)(1111)	Gg T_1T_2	arbitrary	complete	$z_2(a + e, b + f, c + g, d + h)$
(31)(1111)	Gg T_1T_2	arbitrary	$T_2 > 0$	$z_2(a + e, b + f, c + g, d + h) + e_2$
(31)(1111)	Gg T_1T_2	$g > 0$	$T_2 > 0$	$z_2(a + e, b + f, c + g, d + h) + c_2$
(31)(1111)	Gg T_3T_4	arbitrary	complete	$z_2(a + c, b + d, e + g, f + h)$
(31)(1111)	Gg T_3T_4	arbitrary	$T_4 > 0$	$z_2(a + c, b + d, e + g, f + h) + e_2$
(121)(211)	G_1G_2 T_1t	complete	arbitrary	$z_1(d + i, f + g)$
(121)(211)	G_1G_2 T_1t	arbitrary	$T_2t > 0$	$z_1(d + i, f + g) + e_1(d + g, f + i)$
(121)(211)	G_1G_2 T_1T_2	arbitrary	complete	$z_1(a + f + i, c + d + g)$
(121)(1111)	G_1G_2 T_1T_2	complete	arbitrary	$z_1(a + f + g + l, c + d + i + j)$
(121)(1111)	G_1G_2 T_1T_2	arbitrary	complete	$z_1(a + f + g + l, c + d + i + j)$
(121)(1111)	G_1G_2 T_1T_2	arbitrary	$T_2 > 0$	$z_1(a + f + g + l, c + d + i + j) + e_1(s_1, s_3)$
(121)(1111)	G_1G_2 T_3T_4	arbitrary	complete	$z_1(a + d + i + l, c + f + g + j)$
(121)(1111)	G_1G_2 T_3T_4	arbitrary	$T_4 > 0$	$z_1(a + d + i + l, c + f + g + j) + e_1(s_1, s_3)$
(211)(211) cis	G_1g T_1t	$G_2g > 0$	$T_2t > 0$	$z_1(e + i, f + h) + c_1(e + f + h + i)$
(211)(211) cis	G_1G_2 T_1T_2	arbitrary	complete	$z_1(a + e + f + h + i, b + c + d + g)$
(211)(211) trans	G_1g T_1T_2	$G_2g > 0$	$T_2t > 0$	$z_1(b + f + i, c + e + h) + c_1(b + c + e + f + h + i)$
(211)(211) trans	G_1G_2 T_1t	complete	arbitrary	$z_1(d + h + i, e + f + g)$
(211)(1111)	G_1g T_1T_2	arbitrary	complete	$z_1(b + f + h + l, c + e + i + k)$
(211)(1111)	G_1g T_1T_2	$G_2g > 0$	$T_2 > 0$	$z_1(b + f + h + l, c + e + i + k) + c_1(b + c + e + f + h + i + k + l)$
(211)(1111)	G_1G_2 T_3T_4	complete	arbitrary	$z_1(a + d + h + i + k + l, b + c + e + f + g + j)$
(1111)(1111)	G_1G_2 T_1T_2	complete	arbitrary	$z_1(a + c + f + h + i + k + n + p, b + d + e + g + j + l + m + o)$
(1111)(1111)	G_1G_2 T_1T_2	arbitrary	complete	$z_1(a + c + f + h + i + k + n + p, b + d + e + g + j + l + m + o)$
(1111)(1111)	G_1G_2 T_1T_2	$G_2 > 0$	$T_2 > 0$	$z_1(a + c + f + h + i + k + n + p, b + d + e + g + j + l + m + o) + c_1$
(1111)(1111)	G_2G_4 T_3T_4	complete	arbitrary	$z_1(a + b + e + f + k + l + o + p, c + d + g + h + i + j + m + n)$
(1111)(1111)	G_2G_4 T_3T_4	arbitrary	complete	$z_1(a + b + e + f + k + l + o + p, c + d + g + h + i + j + m + n)$
(1111)(1111)	G_2G_4 T_3T_4	$G_4 > 0$	$T_4 > 0$	$z_1(a + b + e + f + k + l + o + p, c + d + g + h + i + j + m + n) + c_1$

at least one A_1 or A_2 child from the mating $A_1A_2 \times B0$). Finally, the condition for selection may be $T_2T_4 > 0$ (as at least one MNs child in the mating Ms/NS × MS/Ns). For brevity the three types of selection for 1111 test factors may be denoted by $T_4 > 0$; T_2 , $T_4 > 0$; and $T_2T_4 > 0$.

Smith (1954) has developed maximum likelihood u scores for separation of the parental recombination values in double intercrosses. This facilitates a test of homogeneity of recombination in mothers and fathers and in many cases simplifies the scoring procedure. The parental scores are confounded if both factors are of type 31 or 121. This approach is also applicable to lods, and results in a considerable simplification of the scores. It is necessary to fix the segregation of the first parent in obtaining the score for the second. The order in which the parents are considered is arbitrary, but the scoring is simpler if the parent with the more restrictive selection of the test factor is taken first. Selection of a family through children not scorable for linkage is equivalent to complete selection for the children scored. With the exception of types (31)(31), (31)(121), and (121)(121), all families of known parental genotype may be scored for each parent separately, and apart from a negligible dependence in T_2T_4 selection the scores are independent (table 2). With T_2T_4 selection, the scores for $T_2 > 0$ and $T_4 > 0$ are formally correct and strictly independent in the absence of linkage. For examples of the procedure see Steinberg and Morton, 1956.

This very simple analysis is all that most data require, and since most families

TABLE 3. DOUBLE INTERCROSS SCORES FOR THE PARENTS JOINTLY

Mating class	Selection		Score	Notes
	G	T		
(31)(31)	arbitrary	$t > 0$	$z_3(a, b + c, d) + e_3$	See tables 12, 13, 17 (Morton, 1955a)
	$g > 0$	$t > 0$	$z_3(a, b + c, d) + c_3$	
(31)(121)	$g > 0$	complete	z_4	For preliminary analysis use $z_3(a + e, b + c + f, d)$
(121)(121)	arbitrary	complete	z_5	For preliminary analysis use $z_3(a + e + g + i, b + c + f + h, d)$
(31)(211)	$g > 0$	$T_2t > 0$	$z_6 + c_3$	
	arbitrary	$T_2t > 0$	$z_6 + e_3$	
(31)(1111)	$g > 0$	$T_4 > 0$	$z_7 + c_2$	
	$g > 0$	$T_2, T_4 > 0$	$z_7 + c_7$	
	$g > 0$	$T_2T_4 > 0$	$z_7 + c_2$	
	arbitrary	$T_4 > 0$	$z_7 + e_2$	
	arbitrary	$T_2, T_4 > 0$	$z_7 + e_7$	For preliminary analysis score parents separately (table 2)
	arbitrary	$T_2T_4 > 0$	$z_7 + e_2$	
(121)(211)	complete	$T_2t > 0$	z_8	
	arbitrary	$T_2t > 0$	$z_8 + e_4$	
(121)(1111)	complete	arbitrary	z_9	
	arbitrary	$T_4 > 0$	$z_9 + e_1(s_1, s_2)$	
	arbitrary	$T_2, T_4 > 0$	$z_9 + e_9$	
	arbitrary	$T_2T_4 > 0$	$z_9 + e_4$	
(211)(211), (211)(1111), (1111)(1111)				Score parents separately (table 2)

TABLE 4. LOD FACTORS AND SCORES

$K = 4\theta_1(1-\theta_1) = PQ$
$L = 4\theta_1(2-\theta_1)/3 = PR$
$M = 4(1-\theta_1^2)/3 = QS$
$N = 4(2+\theta_1-\theta_1^2)/9 = RS$
$P = 2\theta_1$
$Q = 2(1-\theta_1)$

$R = 2(2-\theta_1)/3$
$S = 2(1+\theta_1)/3$
$T = 4(1-\theta_1+\theta_1^2)/3$
$U = 2(1+2\theta_1-2\theta_1^2)/3$
$V = 2(1-2\theta_1+2\theta_1^2)$
$W = 4(3-2\theta_1+\theta_1^2)/9$
$X = 4(2+\theta_1^2)/9$

$$z_1(a+d, b+c) = \log \tfrac{1}{2}\{P^{a+d}Q^{b+c} + Q^{a+d}P^{b+c}\}$$

$$z_2(a, b, c, d) = \log \tfrac{1}{2}\{R^aP^bS^cQ^d + S^aQ^bR^cP^d\}$$

$$z_3(a, b+c, d) = \log \tfrac{1}{4}\{W^aL^{b+c}Q^{2d} + 2N^aT^{b+c}K^d + X^aM^{b+c}P^{2d}\}$$

$$z_4 = \log \tfrac{1}{4}\{M^aP^{2b}L^cQ^{2d}T^eK^f + 2T^{a+e}K^{b+d}U^eV^f + L^aQ^{2b}M^eP^{2d}T^eK^f\}$$

$$z_5 = \log \tfrac{1}{4}\{Q^{2u+w}P^{2v+w}V^i + 2K^{u+v+i}V^w + P^{2u+w}Q^{2v+w}V^i\}$$

$$z_6 = \log \tfrac{1}{4}\{R^{a+e}P^{b+c+f}Q^{2d+f}T^e + R^{a+e}P^{b+d+e}T^eQ^{d+2f} + S^{a+e}Q^{b+d+e}T^eP^{d+2f} + S^{a+e}Q^{b+c+f}P^{2d+f}T^e\}$$

$$z_7 = \log \tfrac{1}{4}\{M^aP^{2b+d+f}T^{c+e}Q^{d+f+2h}L^g + T^{a+g}P^{b+2f+h}Q^{b+2d+h}L^eM^e + T^{a+g}Q^{b+2f+h}P^{b+2d+h}M^eL^e + L^aQ^{2b+d+f}T^{c+e}P^{d+f+2h}M^g\}$$

$$z_8 = \log \tfrac{1}{4}\{P^{a+d+f+h+2i}Q^{c+d+f+2g+h}V^e + Q^{a+d+f+h+2i}P^{c+d+f+2g+h}V^e + P^{a+c+2f+g+i}Q^{c+2d+c+g+i}V^h + Q^{a+c+2f+g+i}P^{c+2d+c+g+i}V^h\}$$

$$z_9 = \log \tfrac{1}{4}\{P^{a+c+2d+e+h+2i+j+l}Q^{a+c+e+2f+2g+h+j+l}V^{b+k} + Q^{a+c+2d+e+h+2i+j+l}P^{a+c+e+2f+2g+h+j+l}V^{b+k} + P^{2a+b+d+f+g+i+k+2l}Q^{b+2c+d+f+g+i+2j+k}V^{e+h} + Q^{2a+b+d+f+g+i+k+2l}P^{b+2c+d+f+g+i+2j+k}V^{e+h}\}$$

c_1, c_2, c_3. See table 13 (Morton, 1955a).

$$c_7 = \log \frac{4^{s+1}(4^s - 3^s - 2^{s+1} + 1) + 8(6^s) - 4(3^s)}{4^{s+1}(4^s - 3^s - 2^{s+1} + 1) + 4(6^s)(R^s + S^s) - 3^s(M^s + 2T^s + L^s)}$$

$$d_2(s_1, s_2) = \log \frac{2^{2s+1} - 2(3^s)}{2^{2s+1} - 3^s(R^{s_1}S^{s_2} + S^{s_1}R^{s_2})}$$

$$e_1(s_1, s_2) = \log \frac{2^{s+1} - 2}{2^{s+1} - (P^{s_1}Q^{s_2} + Q^{s_1}P^{s_2})}$$

$$e_2(s_1, s_2) = \log \frac{2^{s+1} - 2}{2^{s+1} - (R^{s_1}P^{s_2} + S^{s_1}Q^{s_2})}$$

$$e_3(s_1, s_2) = \log \frac{4^{s+1} - 4(3^s)}{4^{s+1} - 3^s(W^{s_1}L^{s_2} + 2N^{s_1}T^{s_2} + X^{s_1}M^{s_2})}$$

$$e_4(s_1, s_2, s_3) = \log \frac{4^{s+1} - 4(3^s)}{4^{s+1} - 3^s(M^{s_1}T^{s_2}L^{s_3} + 2T^{s_1+s_3}U^{s_2} + L^{s_1}T^{s_2}M^{s_3})}$$

$$e_7(s_1, s_2) = \log \frac{4^{s+1} - 2^{s+3} + 4}{4^{s+1} - 2^{s+2}(R^{s_1}P^{s_2} + S^{s_1}Q^{s_2}) + (M^{s_1}P^{2s_2} + 2T^{s_1}K^{s_2} + L^{s_1}Q^{2s_2})}$$

$$e_9 = \log \frac{4^{s+1} - 2^{s+3} + 4}{4^{s+1} - 2^{s+2}(P^{s_1}Q^{s_3} + Q^{s_1}P^{s_3}) + (P^{2s_1}K^{s_2}Q^{2s_3} + 2K^{s_1+s_3}V^{s_2} + Q^{2s_1}K^{s_2}P^{2s_3})}$$

TABLE 5. POWERS OF LOD FACTORS

$\Theta_1 = .05$

x	K^x	L^x	M^x	N^x	P^x	Q^x
1	.1900	.1300	1.330	.9100	10^{-x}	1.900
2	.03610	.01690	1.769	.8281		3.610
3	.$0^2$6859	.$0^2$2197	2.353	.7536		6.859
4	.$0^2$1303	.$0^2$2856	3.129	.6857		13.03
5	.$0^2$2476	.$0^3$3713	4.162	.6240		24.76
6	.$0^3$4705	.$0^3$4827	5.535	.5679		47.05
7	.$0^3$8939	.$0^3$6275	7.361	.5168		89.39
8	.$0^3$1698	.$0^3$8157	9.791	.4703		169.8
9	.$0^3$3227	.$0^3$1060	13.02	.4279		322.7
10	.$0^3$6131	.$0^3$1379	17.32	.3894		613.1

	R^x	S^x	T^x	U^x	V^x	W^x	X^x
1	1.300	.7000	1.270	.7300	1.810	1.290	.8900
2	1.690	.4900	1.613	.5329	3.276	1.664	.7921
3	2.197	.3430	2.048	.3890	5.930	2.147	.7050
4	2.856	.2401	2.601	.2840	10.73	2.769	.6274
5	3.713	.1681	3.304	.2073	19.43	3.572	.5584
6	4.827	.1176	4.196	.1513	35.16	4.608	.4970
7	6.275	.08235	5.329	.1105	63.64	5.945	.4423
8	8.157	.05765	6 768	.08065	115.2	7.669	.3937
9	10.60	.04035	8.595	.05887	208.5	9.893	.3504
10	13.79	.02825	10.92	.04298	377.4	12.76	.3118

$\Theta_1 = .10$

x	K^x	L^x	M^x	N^x	P^x	Q^x
1	.3600	.2533	1.320	.9289	.2000	1.800
2	.1296	.06418	1.742	.8628	.04000	3.240
3	.04666	.01626	2.300	.8015	.$0^2$8000	5.832
4	.01680	.$0^2$4119	3.036	.7445	.$0^2$1600	10.50
5	.$0^2$6047	.$0^2$1043	4.007	.6915	.$0^3$3200	18.90
6	.$0^2$2177	.$0^3$2643	5.290	.6424	.$0^3$6400	34.01
7	.$0^3$7836	.$0^3$6696	6.983	.5967	.$0^3$1280	61.22
8	.$0^3$2821	.$0^3$1696	9.217	.5543	.$0^3$2560	110.2
9	.$0^3$1016	.$0^3$4298	12.17	.5148	.$0^3$5120	198.4
10	.$0^3$3656	.$0^3$1089	16.06	.4782	.$0^3$1024	357.0

	R^x	S^x	T^x	U^x	V^x	W^x	X^x
1	1.267	.7333	1.213	.7867	1.640	1.249	.8933
2	1.604	.5378	1.472	.6188	2.690	1.560	.7980
3	2.032	.3944	1.786	.4868	4.411	1.948	.7129
4	2.574	.2892	2.167	.3830	7.234	2.433	.6369
5	3.261	.2121	2.630	.3013	11.86	3.038	.5689
6	4.130	.1555	3.191	.2370	19.46	3.794	.5083
7	5.232	.1141	3.871	.1864	31.91	4.739	.4540
8	6.627	.08364	4.697	.1467	52.33	5.918	.4056
9	8.394	.06134	5.699	.1154	85.82	7.391	.3623
10	10.63	.04498	6.915	.09076	140.7	9.231	.3237

TABLE 5—*Continued*

$\theta_1 = .20$

x	K^x	L^x	M^x	N^x	P^x	Q^x
1	.6400	.4800	1.280	.9600	.4000	1.600
2	.4096	.2304	1.638	.9216	.1600	2.560
3	.2621	.1106	2.097	.8847	.06400	4.096
4	.1678	.05308	2.684	.8493	.02560	6.554
5	.1074	.02548	3.436	.8154	.01024	10.49
6	.06872	.01223	4.398	.7828	$.0^{2}4096$	16.78
7	.04398	$.0^{2}5871$	5.629	.7514	$.0^{2}1638$	26.84
8	.02815	$.0^{2}2818$	7.206	.7214	$.0^{2}6554$	42.95
9	.01801	$.0^{2}1353$	9.223	.6925	$.0^{2}2621$	68.72
10	.01153	$.0^{2}6493$	11.81	.6648	$.0^{2}1049$	110.0

	R^x	S^x	T^x	U^x	V^x	W^x	X^x
1	1.200	.8000	1.120	.8800	1.360	1.173	.9067
2	1.440	.6400	1.254	.7744	1.850	1.377	.8220
3	1.728	.5120	1.405	.6815	2.515	1.615	.7453
4	2.074	.4096	1.574	.5997	3.421	1.895	.6758
5	2.488	.3277	1.762	.5277	4.653	2.224	.6127
6	2.986	.2621	1.974	.4644	6.328	2.609	.5555
7	3.583	.2097	2.211	.4087	8.605	3.062	.5037
8	4.300	.1678	2.476	.3596	11.70	3.592	.4566
9	5.160	.1342	2.773	.3165	15.92	4.215	.4140
10	6.192	.1074	3.106	.2785	21.65	4.946	.3754

$\theta_1 = .30$

x	K^x	L^x	M^x	N^x	P^x	Q^x
1	.8400	.6800	1.213	.9822	.6000	1.400
2	.7056	.4624	1.472	.9648	.3600	1.960
3	.5927	.3144	1.786	.9476	.2160	2.744
4	.4979	.2138	2.167	.9308	.1296	3.842
5	.4182	.1454	2.630	.9142	.07776	5.378
6	.3513	.09887	3.191	.8980	.04666	7.530
7	.2951	.06723	3.871	.8820	.02799	10.54
8	.2479	.04572	4.697	.8663	.01680	14.76
9	.2082	.03109	5.699	.8509	.01008	20.66
10	.1749	.02114	6.915	.8358	$.0^{2}6047$	28.93

	R^x	S^x	T^x	U^x	V^x	W^x	X^x
1	1.133	.8667	1.053	.9467	1.160	1.107	.9289
2	1.284	.7511	1.110	.8962	1.346	1.225	.8628
3	1.456	.6510	1.169	.8484	1.561	1.355	.8015
4	1.650	.5642	1.231	.8031	1.811	1.500	.7445
5	1.870	.4889	1.297	.7603	2.100	1.660	.6915
6	2.119	.4238	1.366	.7198	2.436	1.837	.6424
7	2.402	.3673	1.439	.6814	2.826	2.033	.5967
8	2.722	.3183	1.515	.6450	3.278	2.250	.5543
9	3.085	.2758	1.596	.6106	3.803	2.490	.5148
10	3.496	.2391	1.681	.5781	4.411	2.755	.4782

TABLE 5—*Continued*

$\Theta_1 = .40$

x	K^x	L^x	M^x	N^x	P^x	Q^x
1	.9600	.8533	1.120	.9956	.8000	1.200
2	.9216	.7282	1.254	.9911	.6400	1.440
3	.8847	.6214	1.405	.9867	.5120	1.728
4	.8493	.5302	1.574	.9823	.4096	2.074
5	.8154	.4525	1.762	.9780	.3277	2.488
6	.7828	.3861	1.974	.9736	.2621	2.986
7	.7514	.3295	2.211	.9693	.2097	3.583
8	.7214	.2812	2.476	.9650	.1678	4.300
9	.6925	.2399	2.773	.9607	.1342	5.160
10	.6648	.2047	3.106	.9564	.1074	6.192

	R^x	S^x	T^x	U^x	V^x	W^x	X^x
1	1.067	.9333	1.013	.9867	1.040	1.049	.9600
2	1.138	.8711	1.027	.9735	1.082	1.100	.9216
3	1.214	.8130	1.041	.9605	1.125	1.154	.8847
4	1.295	.7588	1.054	.9477	1.170	1.210	.8493
5	1.381	.7082	1.068	.9351	1.217	1.270	.8154
6	1.473	.6610	1.083	.9226	1.265	1.332	.7828
7	1.571	.6170	1.097	.9103	1.316	1.397	.7514
8	1.676	.5758	1.112	.8982	1.369	1.465	.7214
9	1.788	.5374	1.127	.8862	1.423	1.537	.6925
10	1.907	.5016	1.142	.8744	1.480	1.612	.6648

are of tabulated scoring types (z_1, z_2, z_3), it should be satisfactory to score the (31)(121) and (121)(121) types with z_3 as (31)(31), neglecting information given by codominance in order to use tabulated scores for all matings. However, if the data suggest linkage (say if $\Sigma z > 2$), extra effort may be worthwhile to extract every bit of information. To do this, the (31)(121) and (121)(121) matings should be scored efficiently with z_4 and z_5. Some other double intercrosses give more information by considering the parents together instead of separately; this is fully efficient if the recombination value is the same in both sexes. These joint scores are given in Table 3, and may be calculated with the aid of Tables 4 and 5 which may also be used to compute scores for large families beyond the range of previous tables. Finally, families of uncertain parental genotype may be scored from the gene frequencies on the assumption of random mating. For rare dominants, the amount of information can be appreciably increased by computing probabilities of large pedigrees, including doubtful families, but the information given by such families is almost negligibly small in data from only two generations (Finney, 1940).

Incomplete penetrance and disturbed viability are considered elsewhere (Morton, 1955a, page 301).

ALLELES AND PSEUDOALLELES

One of the most important problems in linkage analysis is the detection of allelic and pseudoallelic relationships; this may be done efficiently by taking $\theta_1 = .05$. If

there is evidence for linkage of two genes, and if the data include no certain recombinant, then the hypothesis of allelism may be entertained, especially if the two genes have similar effects. However, there is always a possibility that more extensive observation will reveal recombination, and until this is demonstrated a linkage analysis can only provide a confidence interval of the type $0 \leqslant \theta < \theta'$, where θ' is small. Methods will now be given for evaluating θ'.

All of the matings usable in linkage tests give some information about allelism, but the most important sibships are of the z_1 type, with probability proportional to $\theta^w(1-\theta)^x + \theta^x(1-\theta)^w$; this includes double backcrosses ($a + d = w$, $b + c = x$) and two classes ($b = w$, $d = x$) of single backcrosses. If w or x is zero, there is no certain recombinant, but if both w and x are greater than zero, there are $|\, w\text{-}x \,|$ certain recombinants and the hypothesis of allelism is disproved, assuming legitimacy and no errors of classification. Suppose there is no certain recombinant. If there is complete selection for one or both loci, the score for allelism is

$$z^0 = \log \frac{f(y;\theta)}{f(y;0)} = \log \{\theta^s + (1-\theta)^s\},$$

where $w + x = s$ and z^0 is obtained from z_1 by substitution of $f(y;0)$ for $f(y;1/2)$.

If there is truncate selection for both loci,

$$\log \frac{f(y;\theta)}{f(y;0)} = z^0 + c^0, \qquad \text{where } c^0 = \log \frac{2^{s+1} - 3}{2^{s+1} - 4 + \theta^s + (1-\theta)^s}.$$

If there is arbitrary selection at the main locus and truncate selection at the test locus,

$$\log \frac{f(y;\theta)}{f(y;0)} = z^0 + e^0.$$

If $s_1 > 0$ and $s_2 > 0$, $e^0 = -\log \{1 - \frac{1}{2}\theta^{s_1}(1-\theta)^{s_2} - \frac{1}{2}\theta^{s_2}(1-\theta)^{s_1}\}$, and if $s_1 = 0$ or $s_2 = 0$, $e^0 = -\log \{2 - \theta^s - (1-\theta)^s\}$. If a certain recombinant is observed, $z^0 = \infty$.

A small-sample test of allelism may be carried out sequentially against a fixed alternative θ_1 according to the rule $\log B < \Sigma z < \log A$ ($z = z^0$, $z^0 + c^0$, or $z^0 + e^0$), or against a class of alternatives $\theta' > 0$ as follows. Let $z_i(\theta)$ be the score for the i^{th} family and $Z(\theta) = \Sigma z_i(\theta)$. If there is at least one certain recombinant, we reject allelism and terminate the test. If a certain recombinant is not observed, then the test continues and the interval $\theta < \theta'$ corresponding to $Z(\theta)' < \log \alpha$ provides a confidence interval of strength $1 - \alpha$.

Unless the sample is extremely small, this procedure may be replaced by a still simpler one. For if there is no certain recombinant,

$$Z(\theta) = \log e^{-\theta \Sigma s} + 0(\theta), \qquad (s \geqslant 2)$$

and

$$\theta < \frac{\ln (1/\alpha)}{\Sigma s} \qquad (1)$$

is a confidence interval of strength about $1 - \alpha$ if Σs is large. The matings not

TABLE 6. A COMPARISON OF SMALL- AND LARGE-SAMPLE CONFIDENCE INTERVALS OF THE TYPE $\Theta < \theta'$ WHEN THERE IS NO CERTAIN RECOMBINANT

n = the required number of double-backcross sib pairs under truncate selection at both loci
α = the probability of no certain recombinant in a sample of size n if $\Theta = \theta'$
θ'^* = the large sample approximation to θ'

θ'	θ'^*	$\frac{n}{\alpha = .10}$	$\frac{n}{\alpha = .01}$
.10	.081	15	29
.05	.040	29	58
.01	.008	144	288
.001	.001	1439	2878

scorable as double backcrosses, (31)(31), (31)(121), and (121)(121), may be used to detect recombination, but cannot easily be incorporated into equation (1). Familes of known linkage phase contribute to Σ s without the restriction $s \geqslant 2$.

The accuracy of this large-sample interval may be assessed by determining for different values of θ' and α the number n of families containing no certain recombinant required to make a statement of the type $P\{Z(\theta') < \log \alpha\} < \alpha$ and the large-sample interval (1) that would be formed on the same amount of data. For any type of family, $n = (\log \alpha)/z(\theta')$, and the corresponding large-sample limit is $\theta'^* = -(\ln \alpha)/ns$. Using natural logarithms for $z(\theta')$, $\theta'^* = -z(\theta')/s$, which is independent of α. Table 6 compares the large- and small-sample intervals for double-backcross sib pairs under truncate selection at both loci, which because of small family size and incomplete selection is as unfavorable to large-sample theory as any body of data likely to be met in practice. The absolute error is nearly proportional to θ' and of smaller magnitude. If the data are numerous enough to establish linkage and there is no certain recombinant, the large sample confidence interval (1) will clearly be accurate.

PARTIAL SEX LINKAGE

Homologous loci in the X and Y chromosomes are known in some insects and fish (Haldane, 1936), and reports of X-Y chiasmata and postreduction suggest that partial sex linkage may occur in other organisms (Matthey, 1951). In man, the material basis of partial sex linkage is in dispute. Cytologists have variously asserted that the sex chromosomes form pachytene chiasmata and are sometimes postreduced (Koller, 1937), that they never conjugate in meiosis and are always prereduced (Sachs, 1954), and that there is no Y chromosome (Oguma, 1930). The genetic evidence for partial sex linkage is as controversial, and the claim of Haldane (1936) to have detected this condition is not universally accepted (Woolf, 1953; Neel and Schull, 1954). Objections center about the alternative of sex-biased manifestation (viability, penetrance, and ascertainment), as discussed by Harris (1948). The "indirect" method of Haldane (1936), applicable when linkage phase is unknown, does not discriminate between sex-biased manifestation and linkage, and we must rely on cases where the parental phase can be inferred.

Consider first rare recessive traits when the propositi are offspring of unaffected but closely related parents. It is of course possible that the two recessive genes which

come together in the homozygous progeny are of independent mutational origin or are derived from a more remote, possibly unrecorded, common ancestor. However, if p is the gene frequency and F_i the contribution of the i^{th} common ancestor to the inbreeding coefficient of the progeny, the probability of an affected child is $p^2 + p(1 - p)\Sigma F_i$, so that if the largest term in ΣF_i is considerably greater than p or the other F_i, we shall not often be wrong if we assume that homozygosity is by descent from the nearest common ancestor. Haldane used the fact that, on the hypothesis of partial sex linkage, a gene in the father must be on his X chromosome if inherited through his maternal line and on his Y chromosome if inherited through his paternal line. If there are *a* normal and *b* affected children of the same sex as the paternal grandparent who is related to the mother, and *c* normal and *d* affected children of the opposite sex, and if the sex ratio is unity and penetrance complete, the probability of the sibship will be proportional to $(1 + \theta)^a(1 - \theta)^b(2 - \theta)^c\theta^d$, regardless of viability or ascertainment of abnormals.

If we now consider the possibility of sex-biased manifestation, it is obvious that in many cases (about one-half, if the bias is symmetrical) the association between affection and sex of the child will be in the same direction as expected from partial sex linkage. However, if the parents and immediate ancestors of the affected children are normal, there should be no bias with respect to the way in which the husband is related to his wife. This suggests the following test. For a given sibship, fix the numbers of males and females, and of abnormals and normals, and consider the set of two events formed by interchanging *a* with *c* and *b* with *d*. This corresponds to two determinants with the same absolute magnitudes but opposite signs, viz. $\begin{vmatrix} a & b \\ c & d \end{vmatrix}$ and $\begin{vmatrix} c & d \\ a & b \end{vmatrix}$. Under symmetrical sex-biased manifestation, these two events are equally frequent, but if there is partial sex linkage the conditional frequency of the observed type is

$$f(y;\theta) = \frac{(1 + \theta)^a(1 - \theta)^b(2 - \theta)^c\theta^d}{(1 + \theta)^a(1 - \theta)^b(2 - \theta)^c\theta^d + (2 - \theta)^a\theta^b(1 + \theta)^c(1 - \theta)^d}$$

and the lod score for partial sex linkage is $w_2 - z_2$, where

$$w_2 = a \log \{2(1 + \theta_1)/3\} + b \log \{2(1 - \theta_1)\} + c \log \{2(2 - \theta_1)/3\} + d \log \{2\theta_1\},$$

and z_2 is the score for a single backcross of unknown phase. The same linkage score is obtained under asymmetrical sex-bias, if relationships through the husband's male and female lines are equiprobable in the general population of consanguineous marriages. The linkage test is carried out as usual, according to the rule

$$-2 < \Sigma (w - z) < 3.$$

This test is not affected by symmetrical sex-bias regardless of the frequencies of different modes of relationship, nor by asymmetrical sex-bias if relationships through the husband's male and female lines are equally frequent. Unequal frequencies of the two modes of relationship (Morton, 1955b) can simulate linkage only if the same sex preponderates in relationship and affection. Fortunately the recessive factors claimed

TABLE 7. TESTS OF RECESSIVE PARTIAL SEX LINKAGE

Disease	Source	$\Sigma (w - z)$			
		θ_1			
		.1	.2	.3	.4
Retinitis pigmentosa without deafness	Haldane, 1936 Hanhart, 1939	−1.223	.334	.961	.921
Xeroderma pigmentosum	Macklin, 1952 Koller, 1948	−4.991	−2.296	−.816	−.040
Xeroderma pigmentosum	incl. Nerger, 1906	−11.187	−6.039	−3.049	−1.063
Achromatopsia	Haldane, 1936	.871	.809	.696	.474
Oguchi's disease	Haldane, 1936	.252	.400	.415	.298
Spastic paraplegia and ataxia	Haldane, 1941	1.971	1.768	1.448	.912

to be partially sex-linked do not give disturbed sex ratios except for Oguchi's disease, where there is an excess of affected males and of relationship through the husband's male line. None of these factors gives significant evidence for partial sex linkage (Table 7).

The information on which these scores are based is too small to detect or exclude loose linkage, so that nonsignificance of the scores cannot be considered conclusive evidence against partial sex linkage until more data are collected. However, the test is reasonably efficient, with average sample numbers of 22 pairs of affected progeny if $\theta = \theta_1 = .2$, or 45 pairs if $\theta = \theta_1 = .3$. For xeroderma pigmentosum, there is convincing evidence against a value of θ as small as .18, which Fisher (1936b) estimated by the indirect method. Curiously enough, the family of Barckmann (Nerger, 1906), which is contrary to partial sex linkage by the direct test, was the largest contributor to the indirect test. This family was omitted from Haldane's consanguineous matings, apparently because of a misunderstanding of the mode of relationship (Cockayne, 1933, erroneously lists the parents as second cousins). Even without this family, there is no suggestion that xeroderma pigmentosum is partially sex-linked.

An analogous procedure may be followed for rare dominant traits. Only sibships which inherited the gene through the father are informative. If the pedigree contains a normal and b affected children of the same sex as the paternal grandparent who transmitted the gene, and c normal and d affected children of the opposite sex, the score for partial sex linkage is $w_1 - z_1$, where

$$w_1 = (a + d) \log \{2\theta_1\} + (b + c) \log \{2(1 - \theta_1)\},$$

and z_1 is the usual score for a double backcross family of unknown phase. Unfortunately, the method is sensitive to sex-biased manifestation, which also tends to make the sex of affected children concordant with the sex of the affected grandparent. The most that can be expected of this scoring procedure is that it will attenuate the sex bias, since it is not much influenced by exceptional pedigrees, even large ones, the maximum values of $w_1 - z_1$ being log 2 for a single pedigree. However, if the test is significant, it is necessary to apply other methods before the hypothesis of partial sex linkage can be accepted. *Ex hypothesi*, there should be no relation between the

sexes of affected children and affected parents or maternal grandparents, while a positive relation is expected under sex-biased manifestation. Because dominant traits permit examination of affected relatives and no single method is conclusive, tests of partial sex linkage are more eclectic than with recessive traits.

Haldane (1936) proposed that dominant retinitis pigmentosa is partially sex linked in about 40 per cent of the 14 pedigrees he examined. Rywlin (1951) added 11 pedigrees, which showed no evidence for partial sex linkage. Applying my method to the 25 pedigrees, I obtain the following scores

θ	.1	.2	.3	.4
Z	−23.561	−12.489	−5.720	−1.087

The maximum score is .066 at $\hat{\theta} = .481$, which gives no evidence of linkage. The nonsignificance of this test, which does not attach as much weight to large pedigrees as Haldane's test, suggests that the peculiarity of his data is restricted to one or two large pedigrees. His totals clearly indicate the pedigrees responsible. As he pointed out, there is an asymmetrical difference between the progenies of men who inherited retinitis pigmentosa from their mothers and fathers, respectively (Table 8). Men who inherited the condition from their mothers give an excessive number of normal male children with no excess of affected females, and men whose inheritance is from their fathers show an excess of affected male children with no excess of normal females. Moreover, the excesses are largely due to two pedigrees, Bell 5 and 6, which can only be described as extraordinary under any simple genetic hypothesis. The first gives 28 affected children to 3 normal from informative backcross matings, and the second, 25 normal to 2 affected. The extreme asymmetry of the distributions does not in the least suggest partial sex linkage. With these two pedigrees omitted, Haldane's data are no longer significantly in favor of partial sex linkage, and when pooled with Rywlin's pedigrees give an estimated recombination fraction of 164/343 = .478, nearly identical to the value obtained by the w-z scores with Bell 5 and 6 included.

In addition to the nonsignificance of the w-z test and the asymmetry of the two exceptional pedigrees, there is another reason for dismissing evidence based on two peculiar pedigrees out of 25. "It is, of course, characteristic of these linkage studies that large families ··· supply far more information than smaller families. In the absence of methods of collection directed, critically, with a view to the requirements of genetic problems, this constitutes a serious weakness in the available data; for

TABLE 8. DATA ON DOMINANT RETINITIS PIGMENTOSA

Sex of children	Status of children	Haldane (1936)				Rywlin (1951)	Probability under partial sex linkage
		Grandfather affected		Grandmother affected			
		Bell 6	Others	Bell 5	Others		
Same as father's affected parent	affected	19	31	2	29	24	$(1 - \Theta)/2$
	normal	1	26	6	31	33	$\Theta/2$
Opposite	affected	9	21	0	30	23	$\Theta/2$
	normal	2	24	19	38	33	$(1 - \Theta)/2$

linkage ought not to be postulated on the strength of any single family, the recorders of which may have attached no high importance to the correct statement of the sex of the children. We have, in fact, adequate grounds for asserting linkage only when it appears to be indicated by the concurrent testimony of a number of accurately reported families" (Fisher, 1936b).

Partial sex linkage has been suggested for some conditions on data which do not approach significance. Macklin (1952) refuted an earlier claim (Snyder and Palmer, 1943) that idiopathic convulsive disorder gives some indication of partial sex linkage. One pedigree of dominant ataxia and paraplegia (Schut, 1951) showed 6 recombinants and 13 nonrecombinants with the X chromosome, but Haldane (1941) found 34 recombinants among 67 informative children in pedigrees of dominant spastic paraplegia, and Bell's monograph (1948) gives no hint of partial sex linkage in pedigrees of dominant ataxia. The excess of affected males in zygodactyly is more suggestive of sex-biased manifestation than of partial sex linkage (Pipkin and Pipkin, 1945).

Stephens, Perkoff, Dolowitz, and Tyler (1951) traced the inheritance of a semidominant gene for susceptibility to acute pyelonephritis with nerve deafness in adult males and mild renal infection (pyuria, cylindruria, and positive urine cultures with occasional dysuria) in females and young males. Several females and one male transmitted but did not manifest the trait. The inheritance is suggestive of complete sex linkage, except that among the 39 children of affected males there were two sons who at age 2 and 5, respectively, gave evidence of renal infection, and four normal daughters. Among 83 children of affected women there were 20 affected sons and 23 normal daughters. From this the authors concluded that the trait is partially sex linked, with a crossover value of 15.4 ± 5.8 per cent. An alternative is complete sex linkage, with the exceptional females asymptomatic carriers, and the exceptional males sporadics of different etiology who will not develop the characteristic deafness and acute pyelonephritis or transmit the gene. Several kindred with hereditary hematuria and nerve deafness (Sturtz and Burke, 1956) contain only one child from an affected father, a normal son. Children from affected mothers give the ratios expected from an autosomal or sex-linked dominant, with males more severely affected than females. Without a follow-up study, the evidence of this pedigree is not sufficient to establish partial sex linkage.

In a brief note Kaliss and Schweitzer (1943) suggested partial sex linkage of dominant "hemorrhagic diathesis". This trait is a mixture of genetic entities, with both sex-linked and autosomal sex-biased manifestation (for bibliography see Gates, 1946). The evidence they present is inadequate to discriminate partial sex linkage.

AUTOSOMAL LINKAGE

Usually a linkage test in man requires several investigations to detect or exclude moderate or even close linkage. Under these conditions a sequential test is appropriate and highly efficient. The choice of θ_1 is arbitrary, like the sample number in fixed-sample-size tests, but reasonable values of θ_1 are suggested by the average sample numbers for various types of data (Morton, 1955a). If we distinguish common test factors (ABO, Rh, MNS), less common test factors like Duffy, P, and Lutheran, rare "dominants" like elliptocytosis, and rare recessives like cystic fibrosis of the

pancreas, then the following choices for θ_1 will on the average lead to a decision about linkage within a few investigations:

	θ_1
common × less common test factors	.3
two less common test factors	.2
rare dominant × common test factor	.2
rare recessive × common test factor	.1
rare dominant × less common test factor	.1
rare recessive × less common test factor	.05
two rare factors	.05

Although the usual sequential test defined by $-2 < \Sigma z < 3$ is peculiarly appropriate when the data are collected by stages, a nonsequential test may be preferable if information on a particular pair of loci is virtually nonrecurrent, e.g. elliptocytosis × telangiectasia (Roberts, 1945), or for a review of linkage, or to truncate a sequential test if further collection of data does not seem worthwhile. The appropriate small-sample procedure is to accept linkage at a significance level of .001 or less if $\Sigma z > 3$ and to reject linkage or defer a decision if $\Sigma z < 3$. This significance level holds only for preassigned θ_1 ; clearly the data are nonsignificant if the maximum value of Σz is less than 3, but not conversely. Only in rather vast bodies of human linkage data will a large-sample test be entirely trustworthy.

A heterogeneity test should be performed as soon as linkage has been detected and differentiated from other phenomena with which it might be confounded. Small-sample theory has not been developed, but the likelihood ratio test is usually fairly accurate, and seems to be more reliable than the maximum likelihood test (Morton, 1956). If the data are homogeneous, a maximum likelihood estimate of linkage can be obtained from three neighboring points by simple interpolation, or from the frequency of certain recombinants if θ is small, say $<.05$. In the latter case, an accurate confidence interval may be assigned from binomial or Poisson tables. If θ is larger and the amount of data large, a reliable confidence interval is given by normal theory (Morton, 1956). Where this is not appropriate we may use a normalizing transformation, for an approximate confidence interval, or an exact formula of Haldane and Smith (1947). They showed that the inequality

$$Z' > \log \Lambda - \log A \quad (A > 1)$$

where Z' is a lod score and

$$\Lambda = 2 \int_0^{1/2} \Pi \frac{f(y; \theta)}{f(y; 1/2)} \, d\theta,$$

provides, by projection on the θ axis, a confidence interval of strength at least $1 - 1/A$, without any assumption except that θ is constant. In general Λ must be estimated by numerical integration, but if linkage is highly significant and there is at least one certain recombinant, the maximum value of Σz is

$$\hat{Z} \cong \log \left\{ \frac{\Lambda}{2\sigma\sqrt{2\pi}} \right\},$$

and

$$\log \Lambda \cong \hat{Z} + \log \sigma + .7001.$$

These methods have been used to analyze such of the reported autosomal linkages in man as can be scored by lods. For this it is necessary that both traits depend on single, regular mendelian factors. Polygenic associations, pleiotropy "possibly due to several completely linked genes", and various ill-defined genetic correlations are omitted (for these see Gates, 1954). Except where linkage is suggested ($\Sigma z > 2$) only families of known parental genotype have been scored, distinguishing in pedigree data between families of known and unknown parental phase.

Hogben and Pollack (1935) analyzed 12 families segregating for recessive Friedreich's ataxia and ABO, with no evidence of linkage by Bernstein's method. Fisher (1936a) applied his u scores, remarking that "At this early stage in the search for linkage in human genetics, even small collections of data have a certain importance in supplying experience of the kinds of difficulties and inconsistencies likely to be met with, which, if their presence is not suspected, will be liable to give rise to misleading conclusions. Caution is more than ordinarily necessary in this connection, since methods have been developed on purely theoretical considerations, and without experience of their practical reliability." He found $\chi^2_1 = 2.34$ in favor of linkage ($P = .06$) and $\chi^2_{11} = 29.14$ for homogeneity ($P < .01$). The suggestion of linkage and heterogeneity was entirely due to one family which provided "decisive evidence either of linkage, or of the heterogeneity of the twelve families reported". However, Haldane (1946) noted that in the absence of linkage the probability of one such family among several was not small and that the u scores were apparently unreliable. This is also suggested by the probability ratio analysis. The maximum score $\hat{Z}$ is only .44 and the heterogeneity χ^2_{11} only 5.51 ($P > .9$), giving no hint of heterogeneity or linkage. Steinberg and Morton (1956) presented another example of apparently unreliable u scores.

During the last twenty years several linkages have been suggested on nonsignificant evidence. Finney (1940) found a t of 1.70, $P = .045$, for linkage between allergy and ABO, assuming a model for the inheritance of allergy that has not been generally accepted. The probability ratio analysis on the same hypothesis gives $\hat{Z} = .74$, which does not approach significance. Bernstein, Borison, and Finkel (1943) have criticized Finney's claim on other grounds. Burks and Wyandt (1941) found $t = 1.53$, $P = .06$, for linkage between elliptocytosis and ABO, but did not publish the family data. Subsequent studies on elliptocytosis have not suggested linkage with ABO. Bianco, Ceppellini, Silvestroni, and Siniscalco (1954) reported that u scores gave $t = 3.12$, $P < .001$, for linkage between thalassemia and the Lewis^a erythrocyte factor. Through the kindness of Dr. Ceppellini I have been able to apply lod scores to these data, and find $\hat{Z} = .90$. The very slight suggestion of linkage is largely due to a single family, which has probability 1/16 on the null hypothesis. There is also no evidence for linkage between thalassemia and ABO secretion. Snyder (1949) reported linkage between MN and sickling, without distinguishing homozygotes from heterozygotes. As he classified them, his twelve informative families give a χ^2_1 for linkage of 16.64, $P < .001$. Analyzed in the same way, the lod score maximum is 2.26, which is not

significant. His first two families did not segregate for sickling and his four intercrosses contribute no information if homozygotes are sublethal. Omitting these six families, the u-score test gives $\chi^2 = 11.56$, $P < .001$, but the lod score is still nonsignificant ($\hat{Z} = 1.71$). Snyder's data comprise at most 31 units of u-score information. Two later studies, which contribute nearly 300 units, give no evidence of linkage (Waller, Waller, and Hughes, 1952; Neel, Schull, and Shapiro, 1952). This provides a practical demonstration of the reliability of lod scores, the necessity for a stringent significance level, and the unreliability of large-sample linkage tests in man.

Mohr (1954) obtained a strong suggestion of linkage between the erythrocyte antigens Lewis^a and Lutheran ($t = 4.29$, $P < .0001$). Including three additional families (Holt, Thompson, Sanger, and Race, 1952; Mohr, 1956), the maximum lod score is 4.18 at $\hat{\theta} = .06$, and $\Sigma z = 3.17$ against the reasonable alternative of $\theta_1 = .20$. However, there are several reasons for caution in interpreting this result. The evidence for linkage is largely contributed by a single family, number 61, and does not approach significance with this family excluded, although the other families are not significantly discordant with it. Mohr considered Le^a to be recessive, but his families contain an unexpectedly high frequency of children with the antigen ($\chi^2_2 = 5.98$, $P \cong .05$), and the assumed mode of inheritance is probably incorrect (Andresen and Henningsen, 1951). It seems best to be somewhat cautious about this report until studies can be made with Lewis and ABO secretions (Grubb and Morgan, 1949).

Two clear-cut cases of linkage have recently been found by workers at the Galton Laboratory (Renwick and Lawler, 1955). Elliptocytosis is very closely linked to the Rh blood system in four large pedigrees but not linked in three others, which suggests that linkage analysis has subdivided elliptocytosis into two distinct genetic, and presumably phenotypic, entities (Morton, 1956). If this is true, it is the first case in man of the resolution of heterogeneity by autosomal recombination analysis, a method that has had some success for mixed autosomal and sex-linked inheritance in muscular dystrophy, gargoylism, and other traits (Walton, 1955; Herndon, 1954). In families of known parental genotype the cumulative score for $\theta_1 = .2$ exceeds two in the fifth pedigree, and becomes significant in the eighth. The total pedigree score is 7.41, leaving no doubt of the existence of linkage. For the four large pedigrees which show close linkage the estimate of recombination is 3.3 ± 2.3 per cent.

The other proven case of linkage involves the nail-patella syndrome and the ABO blood group (Renwick and Lawler, 1955). Table 9 gives the probability ratio analysis for these data. Heterogeneity is of doubtful significance, in contrast with the maximum likelihood analysis, which erroneously indicates gross heterogeneity between pedigree E and the rest of the data. Renwick and Lawler showed that the P-value for this heterogeneity is greater than .032. The maximum likelihood heterogeneity test may break down when there is no certain recombinant, but the likelihood ratio test does not. Using $\theta_1 = .05$, $\theta_2 = .10$, $\theta_3 = .15$ (Morton, 1956, equation 2), the following estimates are obtained:

$$\hat{\theta} = .110, \sigma = .031, \hat{Z} = 9.62, \log \Lambda = 8.81,$$

which are in close agreement with the values obtained by Renwick and Lawler from more elaborate calculations. A recent pedigree (Jameson, Lawler, and Renwick,

TABLE 9. LOD SCORES FOR LINKAGE OF ABO AND NAIL-PATELLA LOCI
(Renwick and Lawler, 1955)

θ	Complete pedigrees					Total	Families of known parental genotype
	A	B	C	D	E		
.05	1.432	2.912	−1.792	.958	5.301	8.812	2.833
.10	1.430	2.984	−.709	1.117	4.775	9.598	3.802
.15	1.296	2.794	−.177	1.113	4.227	9.253	
.20	1.105	2.488	.092	1.032	3.656	8.374	3.811
.30	.654	1.650	.287	.752	2.446	5.789	2.886
$\hat{\theta}$	1.46	3.01	.30	1.12	5.81	9.62	

Heterogeneity $\chi^2_4 = 9.58$, P ∽ .045

1956) confirms linkage and absence of significant heterogeneity. Most of the information in this record is contributed by 20 double-backcross progeny, with only two certain recombinants. Considering the two lineages from III.4 and III.9 separately, the maximum score is 2.60. Pooling this pedigree with the other five, the corresponding estimates are:

$$\hat{\theta} = .109, \sigma = .028, \hat{Z} = 12.22, \log \Lambda = 11.36, \chi^2_5 = 9.58 \text{ (P } \sim .09).$$

There is no substantial evidence against the hypothesis that the recombination fraction is the same in all six pedigrees. In contrast with elliptocytosis, the nail-patella syndrome appears on present evidence to be controlled by the same locus in different families.

DISCUSSION

Linkage studies in man may be greatly disturbed by genetic heterogeneity, nonrandom assortment of chromosomes, chromosomal aberrations, heritable and environmental variations in recombination rate, and so forth. However, there is no strong evidence to suggest that lod scores will not give reliable answers when applied to appropriate test traits. The utility of linkage analysis is obvious, since the first established autosomal linkage apparently discriminates between two previously unrecognized genetic entities. Such studies will ultimately provide a sounder basis for estimates of single-locus mutation rates, gene frequencies, and selection effects, as well as for the classification of medical entities. Linkage maps may also be of occasional use in the detection of genetic carriers. The problem remains of deciding what markers to use, how much data to collect, and how to do it most efficiently.

A relevant datum is the probability of detecting linkage in routine tests. Let us suppose that human autosomes are about 100 centimorgans in lengths, in which case the recombination fraction θ will have a nearly uniform distribution for linked genes, with a probability of about .05 that a random pair of genes be linked (Morton, 1955a). Assume also that the average power for double backcross sib pairs is approximately correct for other types of data (Morton, 1955a, Fig. 5), that the test loci are in different chromosomes, and that θ_1 is chosen as in the previous section (which will often require that several investigations be pooled). This means that if θ is constant,

the probability of detecting linkage to a particular test factor is about (.05)(.28) = .014 for $\theta_1 = .05$, .0195 for $\theta_1 = .1$, .028 for $\theta_1 = .2$, and .0355 for $\theta_1 = .3$ (Morton, 1955a, Table 2). If there are x common test factors and n less-common test factors, the chance of detecting linkage is about D = .028x + .0195n for rare dominant main factors, and about R = .0195x + .014n for rare recessives. Thus with ABO, Rh, and MNS alone, D = .084 and R = .058, but with 8 additional factors (P, Lutheran, Kell, Duffy, Kidd, etc.), D = .24, R = .17. The chance of detecting linkage with rare recessives is not so much less than for rare dominants as might be supposed from the differences in the appropriate values of θ_1. However, heterogeneity of θ tips the balance considerably in favor of rare dominants, since large pedigrees are more likely than small families to permit recognition and an incisive analysis of linkage. For a test against $\theta_1 = .3$ the probability of detecting linkage with double backcross sib pairs is reduced from .0355 per test factor, if θ is constant, to about .028 if for each family nonlinkage and linkage with constant intensity θ are equiprobable (Morton, 1956). Loss of power under genetic heterogeneity is minimized with a large number of markers, since there will be some chance of detecting linkage with more than one of them. Of course, if heterogeneity in the main factor involves many loci, the chance of detecting linkage with any one of them must be very small, except perhaps in large pedigrees.

The probability of detecting linkage in an extensive study of a rare trait is large enough when θ is constant or bimodal so that the discovery of two linkages by the Galton Laboratory in a few tests is no cause for astonishment. It would seem that routine linkage tests could profitably be carried out in any large-scale family study of a rare recessive, and especially of a rare dominant, and that in the absence of a number of very common test factors, less common traits like Duffy and Lewis can contribute considerably to the probability of detecting linkage.

SUMMARY

Lod scores can be applied to multiple allelic intercrosses very easily when the parental sexes are kept separate, and laboriously but more efficiently by joint scores. Allelism tests and confidence intervals are obtained for the case of no certain recombinant.

Scores which discriminate partial sex linkage from sex-biased manifestation do not confirm claims of partial sex linkage. No case is significant, and there is evidence that earlier tests on retinitis pigmentosa and xeroderma pigmentosum confounded sex-bias with partial sex linkage.

No significant evidence is found for most of the reported autosomal linkages. In at least two cases it is apparent that the lod scores are more reliable than large-sample tests. Linkage between the Lutheran antigen and the Lewis or ABO secretions is likely, but more study of the secretions is required. Two established cases of autosomal linkage are elliptocytosis with Rh, and nail-patella syndrome with ABO.

The probability of detecting linkage in random tests with 11 independent test factors may be as much as .24 for rare dominants and .17 for rare recessives. It is suggested that routine linkage tests would be profitable in extensive family studies of rare genes.

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A Genetic Study of β-Aminoisobutyric Acid Excretion[1]

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INTRODUCTION

SINCE THE simultaneous discovery by Crumpler, Dent, Harris, and Westall (1951) and by Fink, Henderson and Fink (1951) that a particular amino acid, β-aminoisobutyric acid (BAIB), may be excreted in large quantities in the urines of certain individuals, several investigators have shown this trait to be characteristic of individuals and largely independent of environmental factors. Harris (1953), using two-dimensional paper chromatograms, classified individuals as "excretors" or "non-excretors" when the intensity of the spot formed for BAIB was visually greater or less than the intensity of that formed for alanine. On this basis he found that the frequency of "excretors" was 9.6 per cent in the United Kingdom and suggested that the trait was due to a single recessive gene. These findings were confirmed by Calchi-Novati and co-workers (1954) in an Italian population. Gartler (1956), although using a more quantitative determination of the excretion of BAIB, namely by measuring the transmission of light through the spot on paper chromatograms, also classified individuals as "excretors" and "non-excretors" in his genetic analysis of BAIB excretion. He concluded that the frequency of excretors was less than 2 per cent and that the inheritance of the trait was compatible with a single recessive gene hypothesis.

The above studies have been faced with the difficulty of studying a trait which is relatively rare in Western European populations. The discovery by Sutton and Clark (1955) that the frequency of excretors is considerably elevated in populations of Mongoloid extraction offers an excellent opportunity to attempt to elucidate the genetic mechanisms involved. Hence, this paper will be primarily concerned with a genetic study of families drawn from a Chinese and Japanese population.

METHODS

The subjects of the present study are individuals of Chinese and Japanese ancestry, most of whom were born in the Orient but who are residing at present in Ann Arbor, Michigan. Among the 91 subjects studied seventeen families are included, 30 children having been produced from these matings. The small number of children reflects the fact that the parents for the most part are still young. No evidence of a difference between Chinese and Japanese with respect to BAIB excretion has been found.

The experimental procedures employed were the same as reported earlier from this laboratory (Sutton and Clark, 1955) and are based on those used by Berry, Sutton, Cain and Berry (1951). One to three first morning urine samples were col-

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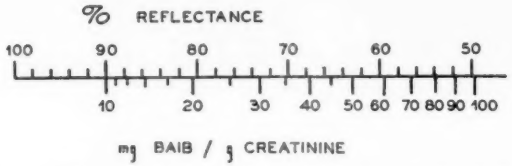

FIG. 1. Scale for conversion of reflectance readings (% R) into mg β-aminoisobutyric acid per g creatinine.

lected from each subject and kept frozen until ready for analysis. Two dimensional paper chromatograms were prepared with an aliquot of urine containing 50 micrograms of creatinine and were resolved with phenol and lutidine as the two solvents.[2] The amino acids were made visible by dipping the dried chromatogram through a 0.2 per cent solution of ninhydrin in 95 per cent ethanol, followed by heating for 7 minutes at 95°C. The heating is essential since BAIB, unlike many other amino acids, does not react appreciably with ninhydrin at room temperature.

The amount of the amino acid present was estimated by measuring the reflectance of the colored spot with a Beckman Model B reflectance attachment, using a colorless portion of the chromatogram to adjust the instrument to 100 per cent reflectance. Throughout the present report the reflectance values (R) are used. It will be seen from the conversion scale in Figure 1 that the reflectance scale is a logarithmic transformation of the absolute values. An R of 100 corresponds to no excretion and an R of 50 to very high excretion.

RESULTS AND DISCUSSION

The independence of BAIB excretion of age and sex has been observed by other investigators in populations with a low frequency of excretors (Harris, 1953; Gartler, 1956). In a group of 69 individuals of Oriental extraction studied in this laboratory the coefficient of regression of BAIB excretion on age was found to be −0.022, which is not significantly different from zero. The age range included was 1 month to 60 years. Likewise, no association with sex was detected.

That diet is of little importance in determining BAIB excretion is demonstrated by the absence of significant correlation ($r = -0.208$) between the 17 husband and wife pairs, many of whom were high excretors. This again is consistent with results obtained by other investigators.

The existence of naturally occurring discrete categories of individuals has been a matter of considerable discussion and conjecture. Gartler (1956) was unable to detect any bimodality in his population, but so few high excretors were present that the chances of demonstrating bimodality were small. In Figure 2 the population distribution is shown for the Japanese and Chinese populations which we have studied. Since the 30 children are excluded none of the individuals represented is related. Although there are a large number of high excretors present, no evidence is provided that individuals fall into discrete categories. It would seem necessary, therefore, in any genetic analysis of BAIB excretion to treat the excretion rate as a continuous variable. It is interesting to note that in 64 individuals from whom three urine samples were obtained and whose mean R fell between 86 and 44 the average stand-

[2] In the case of very young children whose creatinine excretion cannot be used as a measure of urine dilution, it has been found that 50 microliters of urine is a suitable test quantity.

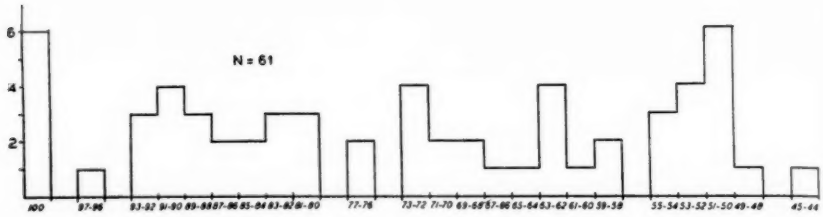

FIG. 2. Distribution of R values for β-aminoisobutyric acid excretion in a population of Chinese and Japanese ancestry.

ard deviation within samples from one individual is 3.28 R units. This indicates the degree of individuality which characterizes this trait.

Among the Chinese and Japanese groups which we have examined were 17 families with one or more children. These have been studied in hope of obtaining addi-

TABLE 1. URINARY EXCRETION OF β-AMINOISOBUTYRIC ACID IN 17 FAMILIES OF ORIENTAL EXTRACTION

Family Number	R* Father	R Mother	Mid-Parent	R Offspring	Sex	Age in Years	Mean Offspring
1	97	53	75	65	M	6	65
2	94	68	81	87	F	9	87
3	87	85	86	92	M	8	89.7
				86	F	7	
				81	F	5	
4	68	97	82.5	85	M	5	85
5	82	79	80.5	73	M	7	73
6	80	71	75.5	88	M	1½	88
7	58	82	70	71	M	7	66
				61	F	6	
8	65	62	63.5	62	M	17	62
9	57	60	58.5	51	F	12	54
				59	F	10	
				52	F	4	
10	71	92	81.5	41	M	2	41
11	91	83	87	100	M	2	100
				100	F	5	
12	84	63	73.5	64	F	5	59
				54	M	2	
13	58	93	75.5	86	F	5	79
				81	F	11	
				84	F	8	
				65	F	3	
14	68	81	74.5	65	M	30	66.6
				67	F	29	
				68	F	11	
15	80	50	65	53	F	8	48
				43	M	5	
16	51	73	62	90	M	4	90
17	100	80	90	81	M	3	81

* R = Per cent reflectance value obtained from two-dimensional chromatograms.

tional information on the genetic mechanisms operating in BAIB excretion. In Table 1 are grouped for each family the following information: excretion rate of both parents, mid-parent value, excretion rate, age and sex of each offspring as well as a mean sibship value. As already pointed out the trait has been found to be independent of age and of sex.

A three-dimensional representation of our family data is shown in Figure 3. Sibs are united by a horizontal line and the length of the vertical segments is proportional to the actual excretion of BAIB (e.g. a long segment corresponds to a high excretor). In the area which corresponds to low excreting parents only low excreting children appear. Conversely the mating of high excretor by high excretor—referring to a range rather than to an attribute—results in a high excretor child. Also, one may notice in sibships of two or more that the values of sibs are similar, thus showing little tendency for segregation.

Further information is provided by measuring the correlation between parents and offspring. The mid-parent/all offspring correlation coefficient is 0.531 while the mid-parent/mean offspring correlation coefficient is 0.463. Although these coefficients are obtained from a different number of "points" on the diagram, namely, 30 in the first case and 17 in the second, the number of degrees of freedom should be chosen in both cases as $17 - 2 = 15$. Thus the mid-parent/all offspring correlation coefficient is significantly different from zero at the 0.03 level and the mid-parent/mean offspring at the 0.06 level. The fact that both coefficients are not significantly different from each other reflects the small amount of segregation mentioned earlier.

In an attempt to detect any paternal or maternal predominant effect, correlation coefficients have been computed for each parent separately. r (father/all offspring) = 0.273 with d.f. = 15 is non-significant, while r (mother/all offspring) = 0.518 with d.f. = 15 is significant at the 0.03 per cent level. The reality of this difference may be tested by computing partial coefficients of correlation. Then r (father/all offspring, holding mother constant) = 0.543, d.f. = 14, and r (mother/all offspring, holding father constant) = 0.652, d.f. = 14. These coefficients show no significant difference from each other, indicating no demonstrable maternal effect.

Finally, if the trait is genetically determined, we may expect a higher variance between sibships than within sibships. Unfortunately the number of sibships larger than one is small, only eight such sibships are available in our data. Nevertheless, these families give good evidence of a very much higher variance between than within sibships ($F = 17.56$, d.f. = 7, 13, $P < 0.001$).

The analysis of our data as well as the results published by others support the

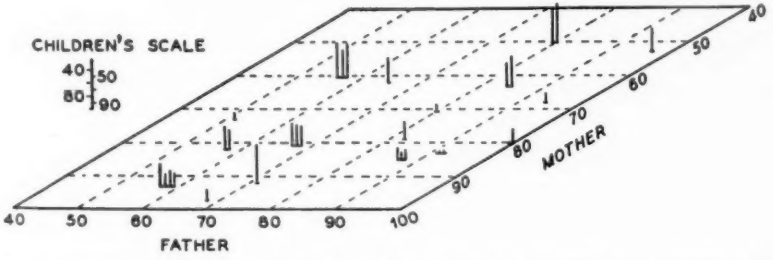

FIG. 3. A three-dimensional diagram of β-aminoisobutyric acid excretion in 17 families of Chinese and Japanese ancestry.

hypothesis of genetic determination of the trait. The correlation that exists between parents and offspring in the absence of a correlation between parents, may only be explained by a genetic mechanism. A difficulty arises, however, when one attempts to elucidate the mode of inheritance. As we have pointed out we find no evidence for classifying individuals into discrete categories which would allow us to test the hypothesis of a single recessive gene.

On the contrary, the fact that the trait is observed to be a continuous variable is hardly compatible with the hypothesis of a two-allele system acting independently of other genetic or environmental factors. The segregation observed in a few families in these and other data (Gartler, 1956) suggests, however, that the major portion of the variation may be produced by the activity of a small number of genes.

In the present study, the subjects have been drawn from a normal population of Orientals. It should be borne in mind that the excretion of BAIB has not been proved to result from identical mechanisms in all individuals. For example, the reported association of BAIB excretion with various pathological conditions should not, on a priori grounds, be assumed to be of identical nature.

SUMMARY

From a study of families of Oriental extraction, support is given to the hypothesis of genetic determination of urinary excretion of β-aminoisobutyric acid. However, the data do not support the hypothesis of a two-allelic system acting independently of other genetic or non-genetic factors. The variation observed may result from a small number of genes.

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Achondroplasia: an Account of the Condition in Northern Ireland

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ACHONDROPLASIA is, superficially at least, an easily defined clinical condition determined usually by a single "dominant" gene. There is considerable diminution of the effective fertility of subjects, probably mainly for social reasons, so that the data from a complete ascertainment have seemed suitable both for direct and indirect calculations of the mutation rate at a locus. The trait has also been suggested as a suitable 'marker' in comparison of populations exposed to different amounts of radiation. The term achondroplasia is used in preference to chondrodystrophy as some writers such as Grebe (1955) include in the latter a variety of dystrophies of cartilages.

It seems fairly clear however, that the etiology of the condition is not as straightforward as at one time appeared. Mørch (1941), accepting all achondroplasia as determined by a single gene, underlined the low viability of subjects recognised at birth and the seemingly normal expectation of life if the subjects survived the first year. He used the frequency in hospital births of achondroplastic subjects born to normal parents to calculate a direct mutation rate and compared this estimate with that derived directly and indirectly from living subjects. It has seemed possible, however, as noted by several commentators (Slatis 1955, and Stevenson 1956) that many of the cases recognised at birth and dying shortly afterwards had a different etiology.

Several families were described by earlier authors where apparently normal parents had more than one affected offspring. These were reviewed by Mørch (1941) and in most accounts the clinical descriptions left considerable doubt as to the true diagnosis. However, Helweg-Larsen and Mørch (1950), and Grebe (1952) have since reported well documented families of this pattern. In the latter's family, the parents were full cousins.

It seemed therefore worth while, apart from the inherent interest of data from a 'complete ascertainment' of any trait, to give an account of the cases in Northern Ireland by presenting data collected in parallel fashion to the Danish data (Mørch, 1941). Consequently this paper presents data relating to all the families having living achondroplastic members and also reviews the number and condition of achondroplastic subjects detected in a large series of hospital births.

ASCERTAINMENT

Living Subjects

During the seven years prior to commencing the study, notifications of achondroplasia had been filed as they were received from a variety of sources. Early in 1955 it was decided to complete the ascertainment. This was done by cooperation of the Medical Officers of Health, School Medical Officers, and Welfare Officers of Local Authorities, pediatricians and consultants in appropriate specialties. Finally every doctor in general practice in Northern Ireland was consulted. The routine procedures

used in this department have been described in detail elsewhere (Stevenson, 1953, and Stevenson and Cheeseman, 1956).

It is always impossible to be certain that all cases have been discovered, and in this ascertainment the most likely omissions would be adults in country districts who are not currently receiving social assistance or medical attention. Such omisssion must, however, be a rarity, as in each visit, the persons visited and the police have been asked about any small people in the area and such people have been visited. One case was ascertained only by this method but most cases were ascertained from several sources and a considerable number of dwarfs notified proved to be suffering from other conditions. It is possible also that some subjects at the upper limits of the height distribution of achondroplasia which seems to be about 5′ 1″ have been missed. It is notable that in Mørch's and Grebe's series the taller subjects usually appear as parents of achondroplastic children and some may have been ascertained through their offspring.

Children recognised at birth in a hospital

The records of the Royal Maternity Hospital, Belfast covering the period from January 1st, 1938 to June 30th, 1956 were searched and in this way a series of nine subjects recognised at birth were ascertained. All had died prior to the beginning of this study.

TAKING OF HISTORIES AND EXAMINATION OF SUBJECTS

Each living subject was visited and examined in his or her home. All were asked by letter to come to the Institute of Clinical Science for x-ray examination, and as will be seen from table 1, about half attended. It was not felt justifiable, however, to try to persuade those who were not very willing to attend, particularly the old and young who would have to make long journeys.

Similarly the parents of the children ascertained by birth in hospital were visited and an adequate history compiled from parents' statements and hospital and autopsy records.

DATA RELATING TO LIVING SUBJECTS

The relevant data are set out in tables 1 and 2. Thirty-nine families were ascertained. In one family (1) an achondroplastic subject (born to normal and unrelated parents) had married an unaffected woman and had had eight children, two being achondroplastic. In 36 of the families (2–37) there was in each only a single achondroplastic subject born to normal parents and there was no history of the condition in the family. None of these 36 affected subjects, who reached appropriate ages, were married or were known to have had offspring out of wedlock. In none of these 36 families were the parents of the subject known to be related. There were in all therefore 37 affected subjects who may be presumed to have received a fresh mutation.

In the two remaining families (38 and 39) (table 2) both sets of parents were unaffected but in each, two offspring were achondroplastic. There was no family history of the condition in these two families but in one (38) the parents were full cousins (their fathers being brothers).

Of the 37 "sporadic" cases (including the father in A1 and excluding families 38 and 39) only three were certainly recognised at birth. The older subjects seldom knew when their condition was recognised and so in fifteen cases there is no evidence, one way or the other. In most of the younger subjects the condition, according to the

parents, was recognised only when the child started to walk or when failure to grow led to consulting the family doctor. Reference to the family doctor and hospital where the child was born confirmed this in many cases. In all, 19 cases were definitely not recognised at birth. The times at recognition, where known, are set out in table 1.

It so happened that none of the living subjects were born in the Royal Maternity Hospital, so that it was not possible to see whether different diagnostic standards prevailed there.

DATA RELATING TO SUBJECTS RECOGNISED AT BIRTH IN THE ROYAL MATERNITY HOSPITAL, BELFAST

In the period January 1st, 1938 to June 30th, 1956 there were 31,753 confinements in the Royal Maternity Hospital, Belfast leading to live or still births. In that period, nine infants (two males and seven females) were recognised to be achondroplastic at birth, all being born to unaffected and unrelated parents. The relevant clinical and genetic information is set out in table 3.

It is of interest that six of the mothers were admitted because an x-ray diagnosis of achondroplasia had been made before birth ((H)3, 4, 5, 7, 8 and 9). One ((H)2) was admitted because of hydramnios and a previous anencephalic birth. Four of the pregnancies ((H)2, 4, 5, and 9) were complicated by hydramnios. Three of the children were stillborn ((H)2, 6 and 8). Those who were born alive died at 15 minutes, 30 minutes, 5 hours, 12 hours, 5 days and 18 months. The child who lived to 18 months was always weakly, never walked or erupted any teeth and died of bronchitis. Seven of the 9 children came to autopsy. One ((H)6) had cleft palate and polydactyly; one ((H)7) had cleft palate, polydactyly and syndactyly, but none had gross internal anomalies.

Such findings suggest marked selection of these cases for birth in hospital. (Only about 50% of children in Belfast are born in hospital, the basis of selection being primiparity, multiparity (women who have had many children), emergencies, and anomalies of pregnancies detected before birth.)

It may be noted that none of the mothers had x-ray examinations early in pregnancy, but only the radiographies at the times noted in table 3. None of the mothers had any fluoroscopic examinations or therapeutic treatment previous to the relevant pregnancy.

DISCUSSION

The data concerning living subjects

In Mørch's data, 16 of the 69 subjects surviving to twenty years of age had had, at the time of the enquiry, offspring whereas, as has been noted, only one Northern Ireland subject had children. The gross frequencies of the condition in the two countries would be expected therefore to be influenced by these differences.

It is convenient in the first instance to ignore families 38 and 39 where two children were born to unaffected parents because there were none such in Mørch's series. Ignoring also the possible bias of differential mortality in the first year of life, there are in the Northern Ireland series 39 living subjects in an estimated population of 1,387,000 or about 28 per million. The comparable figures from Mørch's data are 86 living in a population of 3,793,000 or about 23 per million. A more useful comparison (which gives, by multiplying the figure by ½, , a direct estimation of the mutation rate) is of the number of "presumed mutant" subjects. This is 37 in 1,387,000,

TABLE 1. DATA RELATING TO PRESUMED DOMINANT GENE DETERMINED LIVING ACHONDROPLASTIC SUBJECTS IN NORTHERN IRELAND

Fam. No.	Achondroplastic subjects presumed to have received fresh mutations							Unaffected sibs			Age (years) of parents at birth of subject		No. of years between birth of subject & birth of previous sib.	Notes.
	Sex	Birth rank	Date of birth	Height (cms)	Skull circ. (cms)	X-ray etc.	When recognised	M	F	T	Father*	Mother*		
1	M	6	8.27.94	130	—	No	3 yrs.	5	2	7	36(31)	33(26)	3	Married—has 8 living children, 2 achondroplastic. Sibship includes 1 S.B. Birth rank 7.
2	M	11	12.27.47	100	56	Yes	"Few months"	1	8	9	34(38)	33(40)	1	2 sibs—Females, had pyloric stenosis. 3 miscs. B.R. 5, 6 and 10.
3	F	8	7.20.96	135	—	No	?	1	6	7	66(39)	48(36)	2	
4	F	2	8.21.96	142	—	No	?	1	3	4	46(48)	34(33)	1	
5	M	9	5.11.49	98	56	Yes	1 yr.	2	6	8	59(39)	48(31)	3	
6	M	7	11.16.08	145	—	No	?	4	3	7	43(38)	34(32)	2	Sibship includes 1 male & 1 female S.B. B.R. 5 and 6.
7	F	5	4.24.94	135	60	Yes	?	4	2	6	49(25)	27(22)	3	
8	M	1	3. 3.55	—	—	—	7 mths.	—	—	—	29(28)	23(26)	—	
9	F	1	12.10.20	99	—	Yes	1 yr.	1	1	2	39(27)	32(27)	—	
10	M	9	4.26.93	Now dead		No	?	4	4	8	67(21)	46(22)	3?	Sibship includes 1 female S.B. B.R. 8.
11	F	2	3. 5.22	—	—	No	"Few months"	2	2	4	30(36)	33(35)	9	Sibship includes 1 female S.B. B.R. 3
12	F	1	6. 8.49	—	—	No	14 months	—	—	—	37(22)	33(21)	—	Parents not willing to co-operate.
13	M	1	5. 8.53	—	—	No	"Few months"	—	—	—	32(25)	25(26)	—	Illegitimate. 1 normal older half brother.
14	M	2	8.16.40	—	—	No	2 yrs.	—	3	3	28(40)	27(34)	2	Mentally retarded.
15	F	2	11.16.43	119	57	Yes	9 months	—	1	1	37(22)	35(25)	3	
16	F	4	12.19.16	122	58	Yes	?	4	5	9	39(34)	33(31)	?	
17	F	3	1.21.49	100	55	Yes	1 yr.	2	2	4	41(23)	34(29)	5	
18	F	3	11.11.31	—	—	No	6 mths.	—	2	2	39(33)	31(34)	5	
19	M	6	2.28.46	100	55	Yes	birth	2	8	10	46(31)	31(24)	1	
20	F	1	12. 3.25	—	—	No	?	—	—	—	30(34)	18(34)	—	Illegitimate
21	M	3	12. 6.90	—	—	No	?	5	3	8	30(28)	31(28)	2	
22	F	1	9.30.42	117	55	Yes	15 months	—	2	2	29(32)	30(32)	—	
23	M	1	9. 8.29	133	62	Yes	?	1	1	2	44(22)	30(21)	—	

24	F	11	5.11.47	103	53	Yes	1 yr.	4	9	13	41(33)	37(26)	1	Sibship also includes 2 miscs. B.R. 12 and 15.
25	M	6	11.27.16	—	—	No	Birth	1	6	7	54(29)	36(24)	3	Accidentally drowned Dec. 1955.
26	M	4	6.16.04	128	63	Yes	?	3	—	3	40(45)	37(35)	3	
27	M	7	4.28.04	—	—	No	?	1	5	6	31(35)	35(33)	2	Sibship also includes 1 misc. B.R. 2
28	M	1	7.17.26	131	58	Yes	2 yrs.	5	1	6	23(30)	22(30)	—	
29	M	7	3.19.42	122	57	Yes	1 yr.	4	3	7	42(42)	34(37)	2	Sibship includes 1 male S.B. B.R. 6.
30	F	6	6. 2.36	—	—	No	1 yr.	3	2	5	47(31)	34(33)	2	
31	M	8	10.29.79	124	61	Yes	?	3	4	7	42(22)	38(19)	2	
32	F	4	9.15.78	122	57	Yes	?	2	1	3	52(26)	38(25)	3	Sibship includes 1 female S.B. B.R. 1.
33	M	1	7. 7.99	132	60	Yes	?	3	—	3	31(25)	19(21)	—	
34	M	8	3. 4.74	124	59	Yes	?	6	2	8	42(27)	35(25)	3	
35	M	4	6. 2.38	127	58	Yes	Birth	1	2	3	37(27)	38(26)	7	
36	F	2	5.25.54	—	—	No	2 yrs.	1	—	1	43(25)	36(24)	2	
37	M	3	6.28.07	131	59	No	6 yrs.	1	1	2	34(38)	32(30)	1	

* Figures in brackets are ages of parents of control series subjects—see text.

TABLE 2. DATA RELATING TO TWO FAMILIES IN NORTHERN IRELAND WHERE TWO ACHONDROPLASTIC SUBJECTS WERE BORN TO NORMAL PARENTS

(No affected relatives were known in either family)

Fam. No.	Affected subjects				Unaffected sibs			Note
	(1)		(2)		M	F	T	
	Sex	Date of birth	Sex	Date of birth				
38	M	3.1.25	F	1.31.35	4	—	4	No consanguinity of parents known. One sib still born "normal". One living sib has cleft palate.
39	F	4.18.23	F	6.21.26	3	—	3	Parents full cousins—their fathers being brothers. In normal sibs twin brothers, probably dizygotic.

or about 27 per million for Northern Ireland (including the father in 1) and 74 in 3,793,000 or about 20 per million for Denmark.

Mørch was concerned to show that because of a high mortality of achondroplastics in the first years of life, figures calculated in this way are residual frequencies. He estimated that the survival rate of chondrodystrophics in the first year of life is only perhaps 20 per cent of that of unaffected infants. His contribution was based on the high mortality of cases recognised in hospital and his acceptance of these cases as being of the same etiology as that of his living subjects.

The rather scanty data available in the literature and in the present study, however suggest that the etiology may not be the same in the living subjects as in those recognised at birth and that there is no evidence of a lesser viability at birth or in the first year in the achondroplastic subjects born to matings of achondroplastic and normal parents.

Subjects where the condition was recognised at birth in hospital

Consideration of the subjects born in the Royal Maternity Hospital series shows that they have a very low viability, and there is evidence of malformations in two of the children. When these are considered in association with the high incidence of hydramnios in the pregnancies, the picture presented is that often found in "congenital" (as opposed to hereditary) conditions.

Cleft palate or polydactyly is not reported as a common finding in adult achondroplastics and did not occur in any of the living Northern Ireland cases. Nor are such anomalies reported by Mørch, or Grebe in their living subjects. Achondroplastics as a whole seem strong and sturdy and although chronic rheumatic affections may be more common in adult subjects and spinal lesions from pressure of abnormal vertebrae have frequently been reported, these are late onset troubles.

Consideration of Mørch's data from the Rigshospital, Copenhagen reveals that his cases show a pattern rather similar to that in Belfast. Mørch had seven cases born to normal parents (his cases 108, 110, 111, 115, 116, 117 and 118). Of these one was stillborn, five died within a day or two of birth and one died at 11 months. (Mørch's case 30 survived to 29 years but the condition of the father was not known.) Two of the children had malformations and the child which died at 11 months of bronchitis had always been weakly and had never erupted teeth.

The frequency of the condition in the Royal Maternity Hospital births was 9 in 31,753 or approximately 283 per million. This may be compared with the Rigshospital series of 7 in 94,075 births or 74 per million. However, as already pointed out there is ample evidence of selection in hospital admissions in respect of mothers of achondroplastic babies. To calculate mutation rates from such frequencies is therefore unrealistic. It would be even less sensible to compare the rates in two hospitals.

It has been suggested by several observers, e.g. Haldane (1949), that in the offspring of matings of achondroplastics and normal subjects there is a failure to reach the 1:1 ratio of affected to unaffected subjects to be expected on a single dominant gene hypothesis. As pointed out by Slatis (1955) the selection of data by Haldane may have tended to over-emphasize the deficiency of achondroplastic subjects. Unfortunately Grebe's data are scanty on this point—six affected and five unaffected in the pooled sibships of the offspring of these matings. It is impossible from published data to see whether the deficiency is likely to have been determined, to an undue extent, by matings which resulted in no achondroplastic offspring.

Parental ages

In Table 1, the ages are given of each parent at the time of birth of the 37 affected subjects presumed to have received fresh mutations together with similar information for 37 control subjects matched by year of birth and sex with the affected subjects. No official statistics relating to parental age at birth of a child are available in Northern Irleand so that these controls were drawn from the casualty out-patient attendances at a Belfast teaching hospital; the first persons attending, after a given time, to satisfy the age and sex criteria, were selected to form the control group. In the controls, the average age of father (31.0 years) exceeded that of mother (28.4 years) by 2.6 years with standard error 0.64 years. This paternal excess is compatible with the only relevant data available about married couples in Northern Ireland, namely a difference of average age at marriage, which varied from 2.6 to 3.5 years, the males being older, over the last eight years.

The mean ages of the fathers and mothers of the living achondroplastics at their birth were 40.2 and 33.0 years respectively, giving a paternal excess of 7.2 years with standard error 1.1 years. At the conventional level of significance used throughout this report, $P = 0.05$, the average excess of fathers' over mothers' age is significantly greater for achondroplastic than for control births by 4.7 years with standard error 1.27 giving $P < 0.001$.

These data agree with those of Mørch (1941) and Grebe (1955), and they support Penrose's (1956) contention that paternal age is the variable most closely associated with mutation rates in achondroplasia.

Mørch (1941) regarded the children born of normal parents, and recognised as achondroplastic at birth in hospital, as recipients of the same fresh mutations as those received by living subjects. Unfortunately he gives no data of parental age. Only nine such cases are reported here from the Royal Maternity Hospital, Belfast and, with so few, any conclusions must be tentative. Table 3 sets out the ages of the parents at the birth of these nine subjects with similar information for a control group composed of the next normal birth of similar age and sex in the hospital records. The mean ages of the fathers and mothers at the birth of these nine "affected" infants were 33.3 and 30.1 years respectively, giving a difference of 3.2 years with standard error of 1.58 years. For the controls, the corresponding ages were 33.9 and 29.2 years, giving a difference of 4.7 years with standard error 1.42 years. Again a paternal excess

TABLE 3. CHILDREN RECOGNISED AT BIRTH AS SUFFERING FROM ACHONDROPLASIA AND BORN TO NON-ACHONDROPLASTIC PARENTS

Royal Maternity Hospital, Belfast. January, 1938–March, 1956.

Fam. No.	Sex	Live birth or still birth	Date of birth	Pregnancy order	Birth weight (gms)	Maturity in weeks	Diagnostic Radiographic Exposures during Pregnancy (weeks)	Autopsy	Unaffected sibs			Father		Mother		No. of years since preceding birth	NOTES
									M	F	T	Age at birth of subject* (years)	Height (cms)	Age at birth of subject* (years)	Height (cms)		
(H)1	F	LB	8. 3.38	4	2948	34	32	Yes	5	3	8	35(37)	163	32(35)	157	1	Lived 30 mins Sibs include 1 female S.B. B.R. 7. Cause of stillbirth not known. No abnormality of pregnancy. Normal birth. Length 15″.
(H)2	F	SB	6.26.49	6	2410	39	38	Yes	3	3	6	39(42)	160	33(39)	155	2	Ante partum still birth—no fetal heart at beginning of labor. Sibs include 1 female S.B. anencephalic. B.R. 1. Hydramnios 5th child. Hydramnios 36th wk. this child. Length 15½″.
(H)3	F	LB	9.28.38	2	2835	40	36	No	3	—	3	34(47)	170	22(38)	157	1	X-ray diagnosis breech. Achondroplasia. Always weakly—imperfect—no teeth. Died bronchitis at 18 mths. Length 18″.
(H)4	F	LB	6. 2.53	1	1559	33	33	Yes	1	—	1	21(23)	160	21(22)	155	—	Ante partum x-ray diagnosis. Vertex — achondroplastic. Lived 5 days. P.M. lumen of foramen magnum encroached upon by 2 antero-lateral & 1 posterior protuberance. No hydrocephalus. Length 14″ Hydramnios.

(H)5	M	LB	8.28.54	5	3515	38	34 & 36	Yes	1	3	4	42(32)	180	33(34)	170	2	Ante partum x-ray diagnosis breech achondroplastic. Hydramnios noted 14 days before birth. Lived 15 minutes.
(H)6	F	SB	1.11.38	3	2211	31	None	No	1	1	2	32(32)	170	33(23)	157	12	Premature still birth. Vertex. Had polydactyly & cleft palate. Length 14″.
(H)7	M	LB	11.28.53	2	2608	40	None	Yes	—	1	1	29(33)	155	28(22)	150	1	Ante partum x-ray diagnosis. Lived 5 hrs. Mother had a miscarriage at 12 wks. in 1952. Child 15¼″—cleft palate—double thumbs & great toes. Fusion of 2nd & 3rd toes right foot. These parents were very small, but not achondroplastic.
(H)8	F	SB	2. 6.56	2	4281	40	32	Yes	—	1	1	36(34)	157	36(29)	152	7	Ante partum x-ray diagnosis. Intra-natal still birth. Vertex—achondroplasia. Forceps delivery 18″.
(H)9	F	LB	2. 7.56	2	2381	33	33	Yes	—	1	1	32(25)	163	33(21)	163	1	Ante partum x-ray diagnosis. Lived 12 hrs. Induced labor because of hydramnios at 32 weeks. Sib was female stillbirth died intra partum asphyxia.

* Figures in brackets are ages of parents of 'control' births matched for sex and birth rank—see text.

occurred in each group, but unlike the excess observed for the living affected subjects, that for the hospital series was not significantly different from the control data (difference, 1.4 years, standard error 2.13 years, $0.60 > P > 0.50$).

Accepting the few data available the living achondroplastic subjects had fathers whose average age was materially greater than their mothers, while no such difference in parental age occurred in the hospital series. This observation adds support to the other evidence advanced that the hospital series were either wholly or in part phenocopies rather than recipients of fresh mutations.

INTERPRETATION OF THE DATA

In living families 1 to 37 inclusive the data are in accord with the view usually advanced that the trait is determined by a single dominant gene.

In families 38 and 39 the possible explanations are that there was failure of manifestation of a dominant gene in one parent, that these families represent the results of a mutant which affects at least part of the germinal tissue or that in these families a recessive gene or allele determined the condition of the offspring. It is suggestive of the recessive explanation that in two of the four families where the condition has been reported with adequate documentation the parents were full cousins (the four families being the two here reported and those of Helweg-Larsen and Mørch (1950), and Grebe (1952)). It has been noted that none of the unaffected parents of single affected children reported here, by Grebe, and by Mørch were known to be related. If the recessive hypothesis is the true explanation, it may be supposed, however, that a proportion—possibly small—of the reported cases should be so explained.

In the presumed dominant gene determined cases it is impossible to know, as is usually the case in human genetics, whether the same effect can be produced by mutation to different alleles or at different loci, so that the usual dubiety about a mutation rate per locus arises.

The rate by direct calculation in Northern Ireland of 13.5 per million is not significantly higher than the rate for Mørch's data, 10.0 per million.

Possibly the clinical distinction between similar cases of Morquio's syndrome and achondroplasia is more difficult than is usually assumed, particularly in those cases classed as achondroplasia where there is considerable abnormality of vertebrae. Other conditions are unlikely to give rise to difficulty. The writer could find no clinical differences between presumed dominant and presumed recessive gene determined cases.

Comments have already been made on the 'different' picture presented by the cases reported here and elsewhere which have been recognised at birth. There are four possible explanations of the severity of the cases recognised at birth: they may represent the very severe expression of the same gene as in living cases; they may be determined by mutation to another allele; or mutation at another locus; possibly the best explanation, however, is that they represent phenocopies.

SUMMARY

Data relating to achondroplasia are presented, collected in a manner parallel to those of Mørch (1941) namely, by a complete ascertainment of living subjects, and by study of a series of cases recognised in hospital at birth. It is suggested that it is possible to distinguish three types of achondroplasia on genetic and clinical grounds. (1) A severe and almost invariably fatal condition usually recognised at birth and characterised by the parents being normal physically and of ages comparable to those in the general population. The related pregnancies seem to be complicated to

an undue extent by hydramnios. The achondroplastic subjects have a very low viability and an undue proportion have other developmental anomalies. Such children may represent 'phenocopies' or the expression of a fresh dominant mutation. (2) The most frequently recognised type of achondroplastic seems to be the living subject who does not appear to experience impaired viability at any age although the evidence about viability in the first year of life is scanty. Most such subjects appear to be determined by fresh dominant gene mutations. The mean difference between paternal and maternal ages of these subjects is 7.2 years. This is significantly higher than the comparable figure for the parents of the subjects in (1) above and the mean difference of parental ages at marriage in Northern Ireland. When they have offspring by normal partners, the children are often affected. There is some doubt as to whether the expected proportion of half the offspring are affected but the data are scanty and it is impossible to test the hypothesis that any failure to reach the expected proportion of offspring is determined by a number of the surviving subjects being "phenocopies". (3) There is suggestive evidence that a form of achondroplasia clinically indistinguishable from that mentioned in (2) above is also determined relatively infrequently by a recessive gene mechanism.

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